MICROBIOLOGY

** This course meets the 1 hr. Microbiology requirement for Florida license renewal. **

ZIKA VIRUS: UPDATE AND OVERVIEW

AUTHOR: Dr. Linda Pifer, Ph.D., SM(ASCP), SM(ABB)

COURSE CODE: M024
CONTACT HOURS: 1
COURSE LEVEL: Basic
CE BROKER #: 20-570261

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COURSE OBJECTIVES

At the end of this course you will be able to:

1. Describe where Zika virus was first discovered.
2. Demonstrate knowledge of other viruses in the family to which Zika virus belongs.
3. Recognize species of mosquitoes that vector Zika virus in the continental U.S.A.
4. Elaborate upon how Zika virus can and cannot be transmitted.
5. Describe the pathology caused by Zika virus in the fetus and newborn.
6. Detail the common pathology exerted by Zika virus in the adult patient.
7. Define the more severe pathologic manifestations of Zika virus in adults
8. Specify whether molecular testing is available yet for Zika virus.
9. State which Zika diagnostic test should be performed on patients within the first 2 weeks after the onset of symptoms.
10. Expand upon the role of the Trioplex assay for Zika, Chikungunya and Dengue viruses in the current Zika crisis.
11. Explain where you would send an equivocally positive (+/-) Zika specimen for further testing.
12. Show that you have learned the most authoritative source of safety information with regard to Zika virus, pregnancy, and travel into the "Zika zone".

RIGHTSHOLDER:

Author: Dr. Linda Pifer, Ph.D., SM (ASCP), GS (ABB)

Last Review: October 2019

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Last Revised 04/14/19

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Last Revised 04/14/19

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1.) Where was Zika virus first discovered?
- **A.** in Brazil
- **B.** in Africa
- **C.** in the Florida Everglades

2.) Zika virus is related to Yellow Fever, Dengue, and Chikungunya viruses.
- **A.** True
- **B.** False

3.) Which species of mosquito was **NOT** included in the Zika virus control warning issued by the WHO and the Pan American Health Organization?
- **A.** Culex pipiens
- **B.** Aedes albopictus
- **C.** Aedes aegypti

4.) By which route has Zika virus **NOT** been proven to be transmitted?
- **A.** sexually
- **B.** tears and bodily secretions
- **C.** respiratory route

5.) What kind of pathology does Zika virus cause in the fetus and newborn?
- **A.** microcephaly, mental retardation, cataracts
- **B.** extra fingers and toes, cleft palate
- **C.** urinary tract deformities, heart valve defects

6.) What pathology does Zika virus cause in adults?
- **A.** cardiac irregularities, heart failure, swollen feet
- **B.** fever, rash, Guillain-Barré Syndrome with paralysis
- **C.** enlarged liver and spleen, vomiting

7.) Other serious symptoms that ZIKV may cause in adults might include:

- **A.** acute disseminated encephalomyelitis and memory loss
- **B.** renal shut down and severe electrolyte imbalance
- **C.** pulmonary failure necessitating a respirator

8.) Molecular testing is available for Zika testing.

- **A.** True
- **B.** False

9.) Real time Reverse Transcription PCR should be performed on serum from persons with symptoms drawn during the first two weeks after the onset of symptoms.

- **A.** True
- **B.** False

10.) The Trioplex assay for Zika, Chikungunya and Dengue viruses has not been authorized for use for general use in the diagnosis of Zika virus infection, but may be used in emergencies.

- **A.** True
- **B.** False

11.) There is no point in sending specimens with equivocal test results to the CDC because they do not have the capability of performing more sensitive and definitive tests upon these samples.

- **A.** True
- **B.** False

12.) Which of the following statement is true?

- **A.** The CDC's travel advisory should be checked before traveling into the Zika zone.
- **B.** Couples considering pregnancy should keep a close watch on CDC advisories if they live in or near the Zika zone or plan to travel there.
- **C.** Both statements are true

******END OF QUIZ******

ABOUT THE AUTHOR

Meet Dr. Linda L. Williford Pifer, PhD, SM (ASCP), GS (ABB). She is currently a professor with the Department of Clinical Laboratory Sciences at the University of Tennessee Health Science Center in Memphis, TN, where she teaches virology, parasitology, human genetics, laboratory safety, and 7 research courses to clinical laboratory science students. In addition, she teaches infectious diseases, genetics, and immunology in the physician assistant program in the College of Medicine and HIV, bioterrorism and emerging infectious diseases in the College of Dentistry.

Dr. Pifer is a graduate of Ole Miss and has earned her doctoral degree in Microbiology at the University of Mississippi Medical Center at Jackson, Mississippi. She spent six years at St. Jude where she and her team were first to successfully cultivate *Pneumocystis jiroveci in vitro* at the height of the HIV/AIDS epidemic. She was also a member of the original NIH AIDS Working Group and established the diagnostic Virology Laboratory at Le Bonheur Children's Hospital. Dr. Pifer has over 60 peer-reviewed scientific publications, several of which have appeared in the Yearbooks of Pathology, Clinical Pathology, Cancer and the Pediatric Digest.

Table of Contents

INTRODUCTION 1

ZIKA VIRUS TIME LINE 1

HISTORIC IMPACT OF ZIKA VIRUS 3

HOW ZIKA VIRUS IS TRANSMITTED 4

PATHOLOGY OF ZIKA VIRUS IN THE FETUS AND NEWBORN 4

PATHOLOGIC IMPACT OF ZIKA VIRUS ON ADULTS 6

LABORATORY TESTS FOR ZIKA VIRUS 7

TRAVELING IN "ZIKA ZONES" 7

PREVENTION OF ZIKA VIRUS INFECTION 8

PROSPECTS OF A ZIKA VIRUS VACCINE 9

INTRODUCTION

Few issues continue to validate the vital role of continuing education for laboratory professionals as much as the steady emergence of new infectious diseases. Legionnaire's disease was documented in a now-famous Philadelphia, PA hotel incident in the 1970's followed by HIV-1 and 2 which emerged from a specific type of chimpanzee in Cameroon, West Africa in the 1980's. Hantavirus, which originated in deer mice, made its presence known in the Four Corners of the desert Southwest in the U.S. on Navajo Indian reservations in the 1990's, prompting an intensive on-site CDC investigation. Other relatively newly recognized respiratory infectious diseases include SARS (Severe Acute Respiratory Syndrome) transmitted by civet cats, swine and bats in the wet markets of Indonesia and camel-borne Middle Eastern Respiratory Syndrome (MERS). These were followed by the ravages of several strains of the horrific Ebola virus raging in Guinea, Sierra Leone and surrounding countries in Eastern Africa. Although none have matched or even approached the worldwide impact of the retroviruses, Zika virus is unique in its potential for widespread pathology due to its hardy vector, the mosquito, plus its ability to disseminate as a sexually-transmitted infectious disease. Particularly disturbing is its propensity for inflicting devastating, irreparable damage upon unborn infants and leaving a small percentage of adults with permanent partial paralysis, visual, memory and concentration problems. These latter three factors guarantee that knowledgeable laboratory professionals will need to be ready to meet the challenge implicit in the increased demand for testing that is on the horizon.

ZIKA VIRUS TIME LINE

Among the countless viruses that have arisen on the African continent, we have also become familiar West Nile Virus that is now found in every state of the union, and have had a few cases of the highly publicized Ebola virus imported into the United States. Since Zika virus (ZIKV) is so often associated with South America and Brazil, in particular, many do not realize that ZIKV is "African-born". Its original homeland is in Uganda, in the Zika Forest of Eastern Africa where it was first discovered in *Aedes africanus* in 1947 by Alexander Haddow, a Scottish scientist from the University of Glasgow (1). The Zika virus was also isolated from a rhesus monkey in 1947. ZIKV is an enveloped, single-stranded RNA virus and is classified as a Biosafety level II agent.

As a Flavivirus that is arthropod-borne (Arbovirus), it is also related to the dreaded Yellow Fever virus, Japanese Encephalitis, Chikungunya virus, and Dengue Fever, which is also called "break-bone fever" due to the severe aches and pains that it causes. The very first transmission electron micrograph of the virus was taken by C. Goldsmith at the CDC in 1957 and around this time, the first human cases of ZIKV were reported in Uganda and the United Republic of Tanzania. The host range of ZIKV appears to be limited to humans and non-human primates. (1).

In 1964, a research worker became mildly ill with ZIKV after having been exposed to the agent via needle-stick, providing the direct cause-and-effect evidence that the virus indeed infects humans. We now know that there are hundreds of variants of the virus, possibly allowing for some variation in intensity of illness and teratogenic effects on fetuses (causing congenital malformations) depending to some extent upon which variant is being considered.

During the 1960's through the 1980's, human infections continued as confirmed by the appearance of antibodies in numerous blood samples, as the virus moved into Western Africa. At that time, it was not particularly noteworthy with regard to the degree of illness that it caused. During that interval, it was also found in mosquitoes in Pakistan, India, Malaysia, Indonesia and in other parts of equatorial Asia (WHO) (2). ZIKV made its debut in the Pacific region in the Federated States of Micronesia on the island of Yap in 2008 where there was a substantial outbreak of the disease. Before this happened, there had been only 14 cases reported anywhere on earth. A remarkable 73 per cent of the populace of Yap Island became infected with ZIKV, and evidence showed that this population had never had any prior experience immunologically with ZIKV before, i.e., that it was a brand new viral agent in this region. Since the populations of Africa and Asia had likely been gradually exposed over time to the virus, it was likely vastly underreported due to the similarity of its symptoms to dengue fever (a Flavivirus) and Chikunguya virus (a Togavirus). In 2012, a scientist from the U.S. who was working in Senegal (Africa) contracted ZIKV and became ill. The first documented sexually transmitted case of ZIKV occurred when he returned to CO to his wife. In 2014-15, two separate lineages of the virus were noted, including an Asian and an African one (2). Shortly thereafter, major island groups in the Pacific experienced outbreaks including Easter Island, French Polynesia, the Cook Islands and New Caledonia. In French Polynesia during this

outbreak, two mothers and their newborns were discovered four days after birth infected with ZIKV, strongly indicating that the virus could cross the placenta to attack the fetus. While this outbreak was occurring (May, 2015), over 1000 blood donors without symptoms were found to be positive to ZIKV via PCR, suggesting that it could be transmitted by donated blood, raising a whole new set of serious concerns. Although the Zika "family tree" has divided into an African and an Asian branch, there are variants known as the Nigerian, Malaysian, Cambodian, Panamanian, Puerto Rican, and Mexican, etc. strains that will, as is the case with most RNA-containing viruses, continue to subdivide into further sub-variants that will undoubtedly differ in both antigenicity and pathogenicity.

HISTORIC IMPACT OF ZIKA VIRUS

At this point, ZIKV had become a grave concern. Physicians and scientists began making a connection between the microcephaly seen in newborns and the severe neurological and autoimmune manifestations observed in thousands of suspected adult cases of ZIKV infection. In fact, microcephaly had been observed many years ago in the offspring of Rhesus monkeys infected with the virus during pregnancy, but no great issue had been made of it at the time. This was a warning that appears to have been dismissed too quickly.

On May 7, 2015, the World Health Organization and the Pan-American Health Organization issued an Epidemiological Alert to its member states to communicate the threat, to inform the public and to exercise all possible strategies to reduce the vector of the virus, primarily *Aedes albopictus* and *Aedes aegypti.* These are the same species of mosquito that transmit dengue and Chikungunya viruses. For only the fourth time in its history, the Centers for Disease Control and Prevention (CDCP) issued a Level One Alert (Feb. 8, 2016) about ZIKV; the only other events were for Hurricane Katrina (2005), for H1N1 influenza in 2009, for the Ebola virus epidemic of 2014-15, and now for ZIKV (3).

To the dismay of the International Olympic Committee, these dire announcements came on the cusp of the XXXI Summer Olympiad in Rio de Janeiro, Brazil. This led to calls from some experts to either cancel, delay or move the games to a "Zika-safe zone". However, enthusiasm for the games and a great deal of insect repellent prevailed and the games went on.

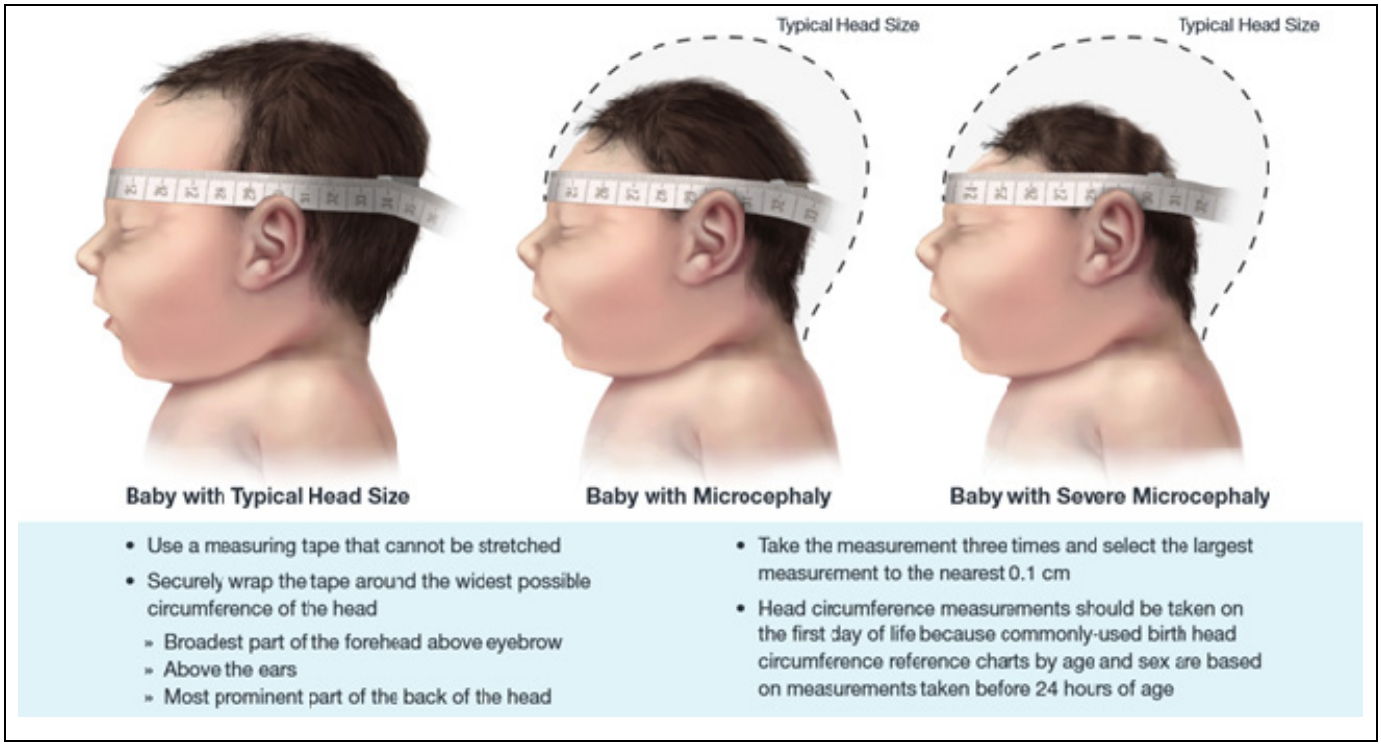
HOW ZIKA VIRUS IS TRANSMITTED

Mosquito-borne ZIKV is the "gift that keeps on giving". Each female mosquito passes the virus on to all of her offspring, thus ensuring the establishment of autochthonous spread, since every female that hatches is already "armed" with the virus and ready to transmit it. Only the female of the species feeds on blood, which is essential to the reproductive process. Male mosquitoes subsist on plant juice. This is an even graver situation than we find in malaria. With regard to the latter, freshly hatched female mosquitoes must first feed on an infected human in order to acquire any of the five species of the *Plasmodium* parasite before they can transmit it to another human. Zika transmitting mosquitoes are born "armed and dangerous" and ready to vector the virus. Then, as if to add insult to injury, we learned that the virus can be transmitted sexually from human to human and also by tears and bodily secretions carrying a very high viral titer. Although the latter type of transmission (tears/bodily fluids) has been documented only once, it emphasizes the fact that we have been continually surprised by this virus and must emphasize safety in the laboratory and not let down our guard. Fortunately, there is no evidence that respiratory or casual contact transmission has ever occurred. It is worth pointing out, however, that the first absolute proof that Zika could infect humans came from a laboratory accident. Further details about transmission will be highlighted in a later section.

PATHOLOGY OF ZIKA VIRUS IN THE FETUS AND NEWBORN

The most vicious and unforgiving impact of ZIKV is upon the fetus of the mother who is infected during her pregnancy. Like cytomegalovirus, other herpes viruses and rubella, ZIKV can cross the placenta and prey directly upon the infant's developing neurons. This is the direct cause of the microcephaly and mental retardation experienced by those so affected. Very recently, using a

mouse model system, Choe and Page reported that ZIKV induces apoptosis (programmed cell death) in neuron progenitor cells that was not observed in older animals. Post-mitotic neurons were also affected with the virus showing a severe impact upon cells that have divided but that are still growing rapidly. Thus, ZIKV has a strong affinity for both rapidly dividing cells and those that are increasing in size (4). The radial glial cells which form a sort of framework for other fetal brain cells fall prey to the virus, causing serious disorientation of the structural foundation of the developing brain. Distinctive features of Congenital Zika Syndrome (CZS) include: severe microcephaly, brain anomalies, ocular findings such as macular scarring, cataracts, swallowing dysfunction, unilateral or bilateral clubfoot, hypertonia/spasticity, hearing impairment and epilepsy. A complete CDC definition of CZS may be found in reference (5). As of Nov. 9, 2016, 2,265 total cases of CZS have been reported with the overwhelming number of these having been identified in Brazil. To date, the USA has experienced only 30 cases (6). However, two of the chief vector mosquitoes for ZIKV transmission are already well-established in North America, and include *Aedes aegypticus and Aedes albopictus*, which does not bode well for the future of an easy task of bringing the virus under control by conventional means involving insect control.



SOURCE: *CDC.gov* **Measuring Head Circumference in Microcephaly**

PATHOLOGIC IMPACT OF ZIKA VIRUS ON ADULTS

In adults, ZIKV causes fever, headache, fatigue, sometimes intensely red eyes, a skin rash and muscle and severe joint pains. In a varying percentage of cases, Guillain-Barre syndrome (GBS) has presented. According to the National Institutes of Health (NIH), GBS is an autoimmune disorder where the peripheral nervous system is attacked by the body's immune defenses. Initial symptoms include a spectrum of weakness or tingling feelings in the legs in some cases, bilateral weakness and odd sensations in the arms and upper body. (7). GBS, however, is definitely not ZIKV-specific and can be triggered by many infectious agents. Recovery may occur in weeks, months or years in extreme cases. Ventilator support may be needed in a few percentage of patients. About 30% may still have symptoms after 3 years.

Another surprisingly severe syndrome associated with ZIKV in adults is acute disseminated encephalomyelitis (ADEM), which was discovered in Brazil and demonstrated that ZIKV could be more dangerous than previously thought. It results in swelling of the myelin sheath of the brain and spinal cord. It was also noted to cause visual, memory and concentration problems (7). In addition to these serious health effects, ZIKV has been shown to remain active and transmissible in semen for up to six months. This presents grave issues to men who have been exposed to the virus and who are wishing to father children. Studies by Lazear et al. (8) in a mouse model showed that ZIKV caused shrunken testes and destruction of their internal structure. Infected males were four times less likely to sire offspring than healthy ones. Thus, it may be that ZIKV in men may ultimately affect their fertility, however, there is insufficient data to draw these same conclusions about human males at this point (8).

There is much yet to be learned about the pathogenesis of ZIKV infection and CZS. One single documented instance was observed where ZIKV was transmitted from a very ill elderly man to his son, ostensibly via tears, perspiration or other secretion, so this does not appear to be a casually transmitted agent (9). Sexual transmission has been documented from male to female, female to male and male to male, even when physiological evidence of infection is absent (9).

LABORATORY TESTS FOR ZIKA VIRUS

Fortunately, molecular testing is available for ZIKV. The CDC recommends that real-time reverse transcription-polymerase chain reaction (rRT-PCF) be performed on persons with symptoms on serum drawn during the first two weeks after the onset of symptoms of ZIKV. Urine should also be collected simultaneously with serum collected less than 14 days after the patient becomes symptomatic. A positive result on either confirms ZIKV, however, a negative rRT-PCR does not exclude ZIKV and testing for anti-ZIKV IgM antibody should be conducted (10). Testing is also recommended for asymptomatic pregnant women who have traveled to active ZIKV areas. RRT-PCR is warranted on serum and urine within two weeks of her last possible exposure. Those coming for testing later than two weeks and who are positive for IgM antibody to ZIKV should also submit serum and urine for molecular testing. Asymptomatic women are advised to have IgM tests done as part of their regular obstetric care (10). The Trioplex Real-time RT-PCR assay has been authorized for use to test for ZIKV under an Emergency Use Authorization (EUA). This test detects Zika, dengue and chikungunya viral RNA, but it *has not been cleared or approved for routine use in ZIKV testing.* ZIKV-specific IgM antibody is generally positive at about four days post-onset of symptoms, and remains detectable for about twelve weeks. If RT-PCR remains negative, antibody testing should also be done for dengue and chikungunya viruses. An ELISA test, the Zika IgM Antibody Capture Enzyme-Linked Immunosorbent Assay (Zika MAC-ELISA) has been used to qualitatively detect ZIKV IgM antibody in both serum and cerebrospinal fluid. However, due to cross-reaction with other flaviviruses, the results may be tricky and difficult to interpret (10). With a presumed positive, inconclusive or equivocal test result, specimens may be sent to CDC or a CDC-designated laboratory for confirmation by the plaque-reduction neutralization test (PRNT). Highly detailed information about ZIKV testing may be obtained at the following web address: http://www.cdc.gov/zika/laboratories/lab-guidance.html.

TRAVELING IN "ZIKA ZONES"

It should also be noted that travel updates regarding ZIKV are issued and updated with great frequency at the following CDC website. Consulting the travel guide is a "must" for those traveling to and from the "ZIKA zones". ZIKV travel

advisories are available at https://wwwnc.cdc.gov/travel/page/zika-travel-information. Recommendations on how long men and women alike should wait before attempting to conceive after having recovered from ZIKV infection are available from the CDC at http://www.cdc.gov/zika/pregnancy/women-and-their-partners.html. Recommendations are given even if one is not sure of having been bitten by mosquitoes while in Zika-positive areas. Highly detailed information about testing pregnant women, infants and children, testing specimens from infants at birth, sexual transmission of ZIKV, dealing with ZIKV and HIV, and understanding ZIKV test results may be found at https://www.cdc.gov/zika/hc-providers/, which is written for laboratory professionals and health care providers. The CDC is your most comprehensive source of expert information about travel, prevention, testing, circumstances of testing, etc.

PREVENTION OF ZIKA VIRUS INFECTION

The thought that a mere mosquito bite could result in microcephaly, mental retardation, etc. in a newborn or permanent neurologic damage in adults is sufficient to rightfully move all residents of ZIKV zones to go to every precaution to avoid mosquito bites. These are severe, life-long impairments. Due to the distribution of various species of mosquito as far north as the Arctic Circle, it will very likely eventually pose a threat to virtually everyone in the continental United States. *Aedes aegypti* and *Aedes albopictus* are, interestingly enough, *daytime and nighttime* mosquitoes, so do not be deceived into thinking that if you stay indoors at night that you will be safe! A trip outside to the mailbox may be sufficient enough to get bitten. Health departments everywhere are recommending mosquito repellants containing DEET, Picaridin, oil of lemon eucalyptus or para-menthane 3, 8-diol and IR3535. This is most definitely not a time to use products that are not recommended by the experts. One should wear long, loose and light-colored clothing with socks. Tucking shirts in pants and pants into socks covered by close-toed shoes or boots affords good protection. One is advised also to avoid perfumes, colognes, and other fragrant products that could entice mosquitoes. The CDC offers detailed information on how to build an "Anti-ZIKV Kit" at http://www.cdc.gov/zika/prevention/prevention-kit.html

PROSPECTS OF A ZIKA VIRUS VACCINE

At the National Institutes of Health (National Institutes of Allergy and Infectious Disease) in Bethesda, Maryland, Phase One clinical trials began on a Zika virus vaccine in September, 2016 including approximately 80 subjects between the ages of 18 and 35 years of age. Testing was initiated at three sites in the continental U.S. including the NIH Clinical Center located in Bethesda, MD. Clinical trials were undertaken to assess the vaccine's safety, efficacy and immunogenicity (11). Other ventures into the vaccine arena are expected by the World Health Organization and some of the major pharmaceutical firms. To be certain, there is no question that there is an urgent and growing need for one, especially when the next generation of infants is at such a dire risk for tragic and irreversible physical and mental disability. Laboratory professionals can make life-changing contributions by providing reliable and accurate information about testing for ZIKV and offering to expedite specimen collection, testing and reporting.

APPENDIX

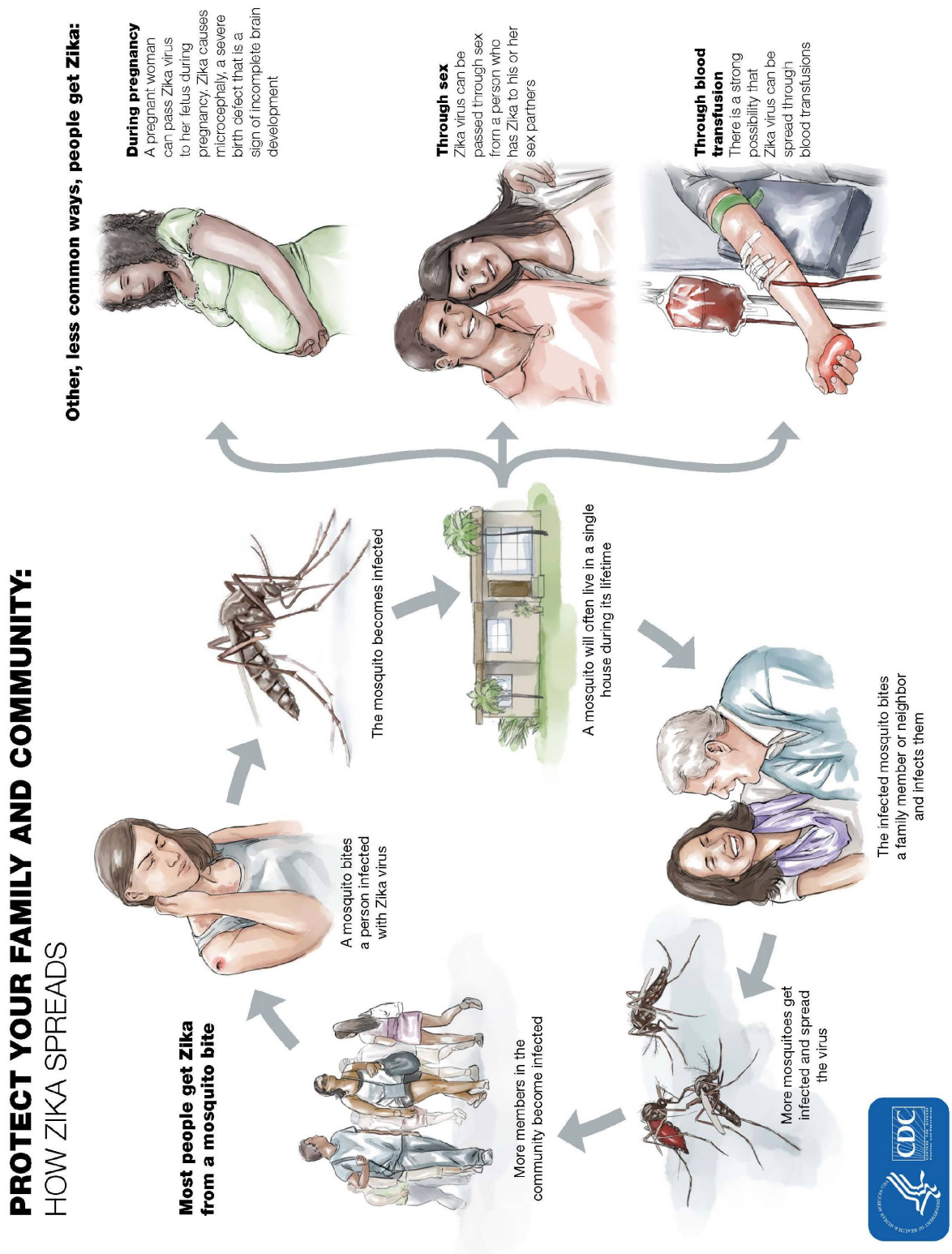
APPENDIX 1 - Modes of Transmission ..... Page 10

APPENDIX 2 – Testing Algorithm for Asymptomatic Pregnant Women ..... Page 11

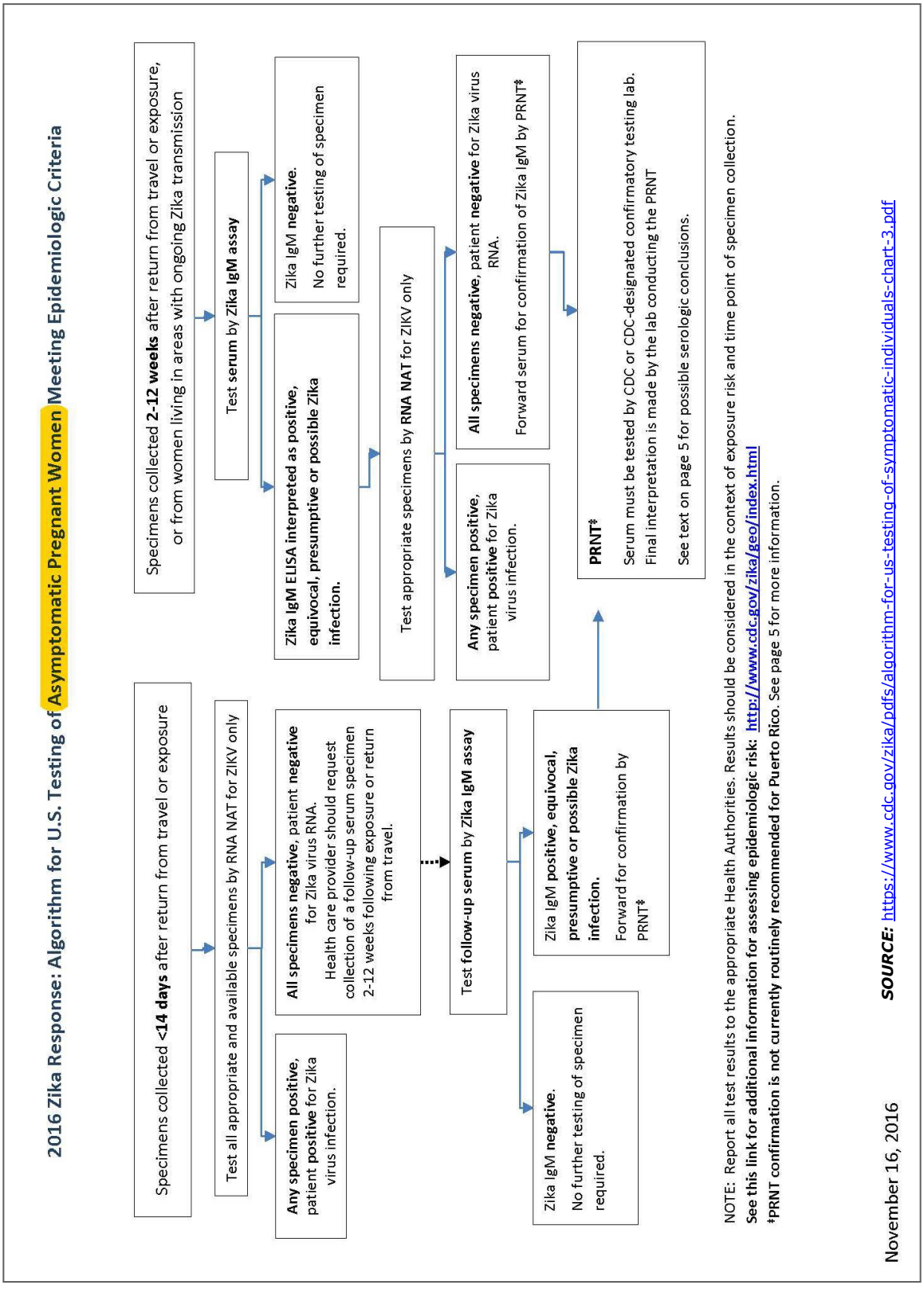
APPENDIX 3 – Testing of Individuals <14 Days Following Symptom Onset..... Page 12

APPENDIX 4 – Testing of Individuals ≥14 Days Following Symptom Onset..... Page 13

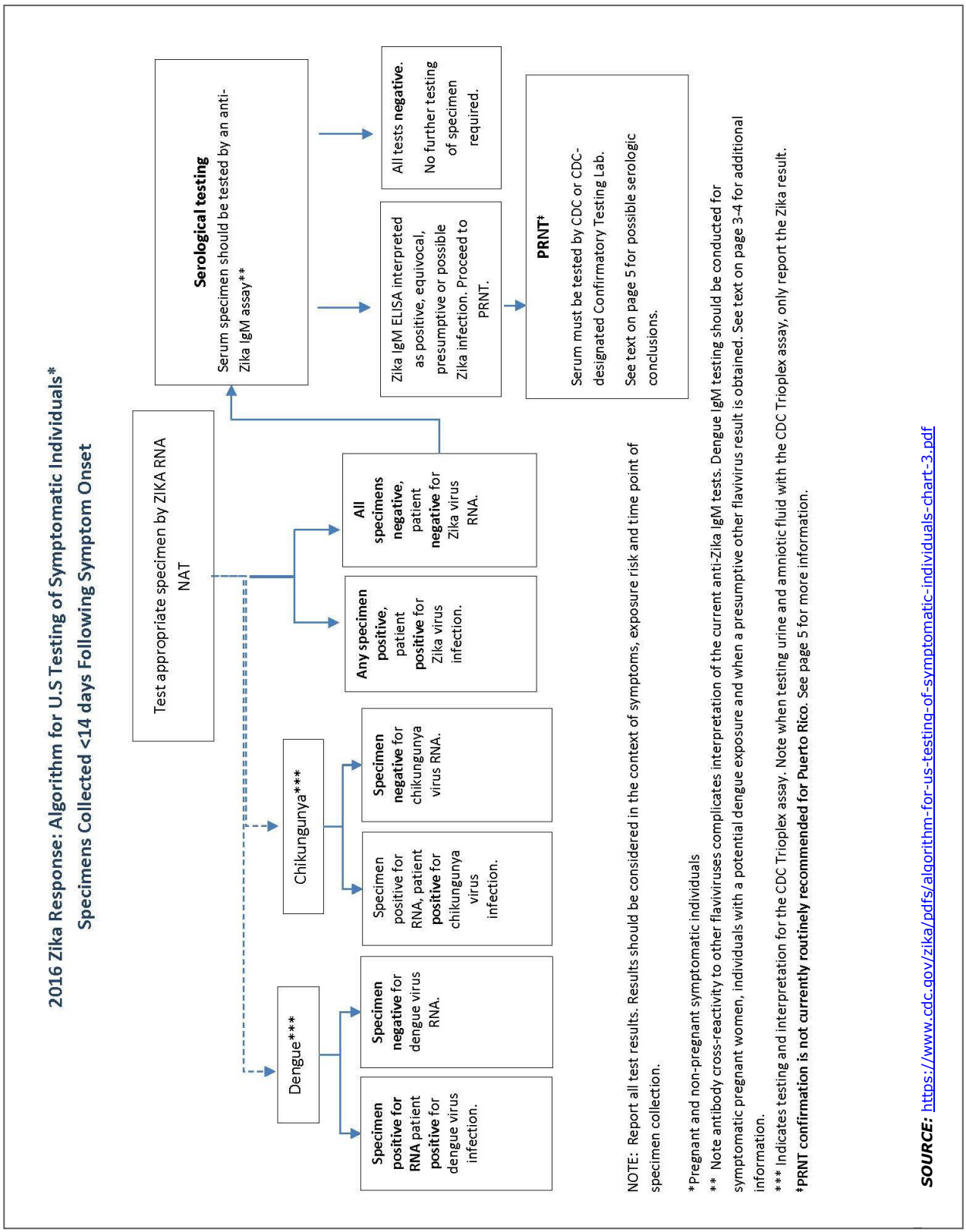
APPENDIX 1 – MODES OF TRANSMISSION



APPENDIX 2 – TESTING ALGORITHM FOR ASYMPTOMATIC PREGNANT WOMEN

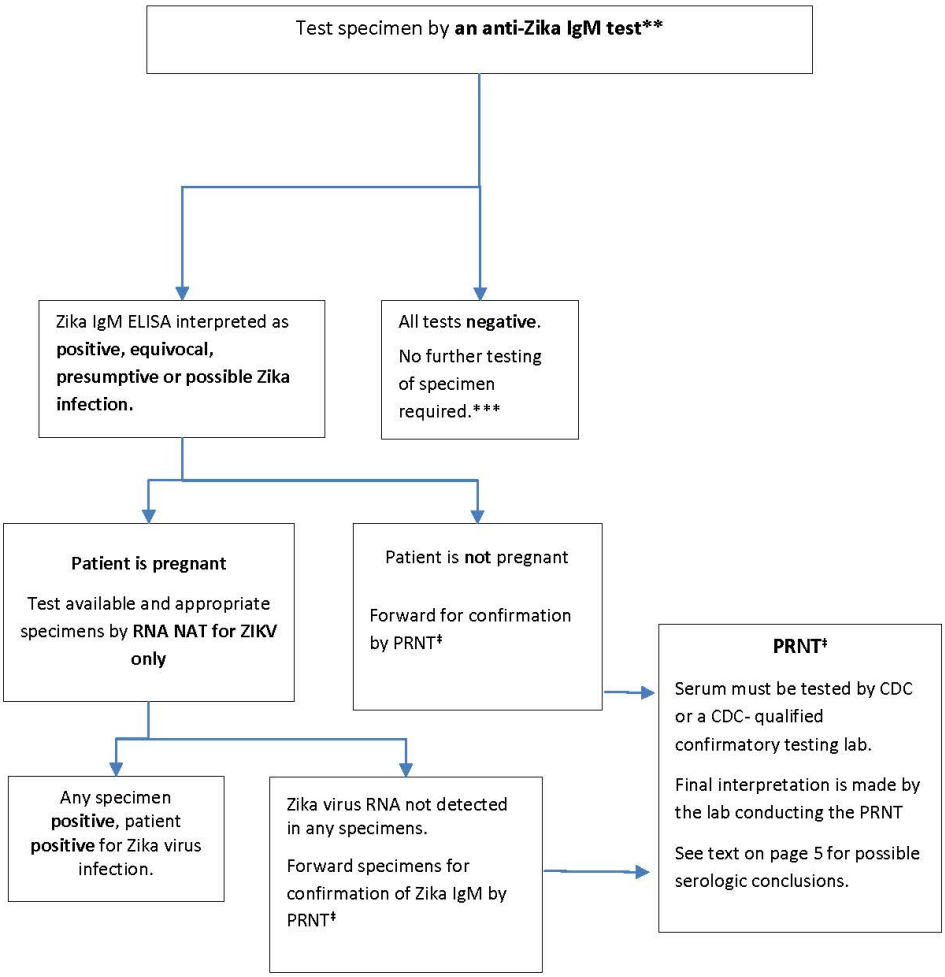


APPENDIX 3 – TESTING OF INDIVIDUALS <14 DAYS FOLLOWING SYMPTOM ONSET



APPENDIX 4 – TESTING OF INDIVIDUALS ≥14 DAYS FOLLOWING SYMPTOM ONSET

2016 Zika Response: Algorithm for U.S. Testing of Symptomatic Individuals*
Specimens Collected ≥ 14 Days Following Symptom Onset



NOTE: Report all test results to the appropriate health authorities. Results should be considered in the context of symptoms, exposure risk and time point of specimen collection.

*Pregnant and non-pregnant symptomatic individuals

**Note antibody cross-reactivity to other flaviviruses complicates interpretation of the current anti-Zika IgM tests. Dengue IgM testing should be conducted for symptomatic pregnant women, individuals with a potential dengue exposure and when a presumptive other flavivirus result is obtained. See text on page 3-5 for additional information.

***Note if tests for Zika and Dengue IgM are not reactive, anti-chikungunya IgM testing should be performed for persons with chikungunya exposure risk and a clinically compatible illness.

‡**PRNT confirmation is not currently routinely recommended for Puerto Rico.** See page 5 for more information.

SOURCE: https://www.cdc.gov/zika/pdfs/algorithm-for-us-testing-of-symptomatic-individuals-chart-3.pdf

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