

Zika Virus!

Report on suspicion of infection during business hours

PROTOCOL CHECKLIST

- Enter available information into Merlin upon receipt of initial report
- Review background information on the disease (see [Section 2](#)), case definitions (see [Section 3](#)), and laboratory testing (see [Section 4](#))
- Forward specimens to the Florida Department of Health (DOH) Bureau of Public Health Laboratories (BPHL) for confirmatory laboratory testing (as needed)
- Inform State Arbovirus Surveillance Coordinator on suspicion of locally acquired arbovirus infection, suspect congenital cases, and suspect associated cases of Guillain-Barré syndrome (GBS)
- Inform local mosquito control personnel of a suspected Zika case as soon as possible (if applicable)
- Contact provider or blood bank (see [Section 5A](#))
- Interview case-patient
 - Review disease facts (see [Section 2](#))
 - Modes of transmission, including sexual transmission
 - Ask about exposure to relevant risk factors (see [Section 5. Case Investigation](#))
 - History of travel, outdoor activities, and mosquito bites two weeks prior to onset
 - Sexual transmission risk and partner's travel
 - Collect pregnancy status and related questions
 - History of febrile illness or travel for household members or other close contacts in the month prior to onset
 - History of previous arbovirus infection or vaccination (yellow fever, dengue, Japanese encephalitis)
 - Provide education on transmission and prevention (see [Section 7](#))
 - Awareness of mosquito-borne diseases
 - Drain standing water at least weekly to stop mosquitoes from multiplying
 - Discard items that collect water and are not being used
 - Cover skin with clothing or Environmental Protection Agency (EPA)-registered repellent such as DEET (*N,N*-diethyl-*meta*-toluamide)
 - Use permethrin on clothing according to manufacturer's directions
 - Cover doors and windows with intact screens to keep mosquitoes out of the house
 - Use condoms correctly and consistently or abstain from sex
- Enter additional data obtained from interview into Merlin (see [Section 5D](#))
- Arrange for a convalescent specimen to be collected (if necessary)
- If case is pregnant, follow up with provider to get medical records for the mother (prenatal records, ultrasound reports, birth records) and infant (birth, 2-, 6-, 12-, 18- and 24-month records) (See [Section 6](#))

1. DISEASE REPORTING

A. Purpose of reporting and surveillance

1. To rapidly detect and monitor exotic arboviral disease activity
2. Work with partners to respond rapidly to arbovirus outbreaks
3. Keep public and other stakeholders informed of activity and increased risk
4. Use surveillance data to monitor success of response
5. Characterize risk factors for infection to use for development of targeted preventive messaging
6. Increase awareness of mosquito-borne illness while traveling

B. Legal reporting requirements

Laboratories and physicians are required to report suspected cases to the local county health department (CHD) (Chapter 64D.3, Florida Administrative Code). Reports should not be delayed for final laboratory confirmation. Report any suspected cases during business hours.

C. County health department investigation responsibilities

1. Begin investigation on the same day as notification.
2. Inform mosquito control personnel of a person under investigation (PUI) for Zika as soon as possible (if applicable). See [Section 5](#) for additional information.
3. For local, pregnant, or infant PUIs, contact commercial laboratories as soon as possible and request that the specimen be forwarded to BPHL-Tampa or -Jacksonville for confirmatory testing. Both polymerase chain reaction (PCR) positive specimens and specimens testing positive, equivocal, inconclusive, or indeterminate on the commercial Zika IgM assay should be forwarded. Specimens for non-pregnant PUIs with travel may also be forwarded if available.
4. Rapidly establish patient travel history in the two weeks prior to symptom onset, within the past two years for asymptomatic individuals, or within the past six months for blood donors.
5. Inform State Arbovirus Surveillance Coordinator on suspicion of locally acquired arbovirus infection, suspected Zika associated microcephaly, poor birth outcome, and GBS.
6. Report all confirmed, probable, and suspect cases in Merlin. See the case definition in [Section 3](#) for proper classification. PUIs not meeting case criteria should be reported as not a case. The Florida Confidential Zika Virus Case Report form is available to assist in follow-up and investigation: www.floridahealth.gov/diseases-and-conditions/disease-reporting-and-management/disease-reporting-and-surveillance/_documents/crf-zika-confidential.pdf
7. Note: Imported and locally acquired Zika cases should be reported in Merlin as non-congenital (Merlin disease code=06010) or congenital (Merlin disease code=06012) based on case definition. Both symptomatic and asymptomatic individuals are included under the same disease codes.

2. THE DISEASES AND THEIR EPIDEMIOLOGY

A. Etiologic agents

Non-congenital Zika cases are caused by infection with Zika virus (ZIKV), a Flavivirus belonging to the family *Flaviviridae*. Congenital cases are caused by vertical transmission of ZIKV from a pregnant woman to the fetus in utero.

B. Description of illness

Symptoms generally last for several days to a week. This illness is characterized by rash, fever, arthralgia, and conjunctivitis. Approximately 1 in 5 people are symptomatic, but even asymptomatic infected persons may still be infectious to mosquitoes for approximately 7–10 days post onset. Severe disease requiring hospitalization is uncommon; however, there have been reports of GBS following suspected ZIKV infection. The full spectrum of fetal abnormalities related to congenital ZIKV infection is not entirely known. However, the Centers for Disease Control and Prevention (CDC) has reported a causal link between ZIKV infection during pregnancy and microcephaly. It is also possible for ZIKV to spread from mother to newborn around the time of birth (perinatal transmission). See [Section 6](#) for additional information on congenital ZIKV infection.

ZIKV disease, dengue fever, and chikungunya fever are difficult to differentiate clinically. Co-infections with these viruses can occur due to vector mosquitoes circulating in many of the same regions of the world.

C. Reservoirs

Humans serve as the primary reservoir for these viruses; however, other vertebrates such as non-human primates may also serve as potential hosts.

D. Modes of transmission

ZIKV is mainly spread through the bite of an infected mosquito, specifically the genus *Aedes*. Although mosquito transmission is most common, ZIKV has the potential to spread through in utero, perinatal, or sexual transmission, and rarely through blood transfusions. While ZIKV has been found in breast milk, ZIKV transmission through breast milk has not been confirmed. The risk of a mother transmitting the virus to her newborn through breast milk is considered low, and the health benefits of breastfeeding greatly outweigh the likelihood of disease transmission. Breastfeeding mothers should consult with their pediatrician about concerns they have regarding breastfeeding and ZIKV risk.

Mosquitoes – *Aedes aegypti* and *Aedes albopictus*

The primary vector for these viruses is *Aedes aegypti*. *Aedes albopictus* is the other important vector and has also become established in Florida. Both species prefer to feed during the day. *Ae. aegypti* feeds almost exclusively on humans, is highly domesticated (evolved to live around homes), and primarily utilizes artificial containers as larval habitats. In contrast, *Ae. albopictus* is an opportunistic feeder and utilizes both natural and artificial containers as larval habitats. Because *Ae. albopictus* feeds on many different animals, risk of infection of humans is reduced compared to *Ae. aegypti*.

E. Incubation period

The incubation period for ZIKV is approximately 2–14 days.

F. Period of communicability

People can transmit the virus to mosquitoes if bitten while viremic; the viremic stage usually begins the day before symptom onset and may continue for about seven days. In some cases, viral RNA may be detectable longer than a week, but it is unknown if enough live virus is present to infect mosquitoes.

G. Treatment

There is no specific treatment for ZIKV disease. Treatment is supportive and aimed at decreasing the severity of symptoms.

H. Prophylaxis

There are no licensed vaccines currently available for ZIKV, although there are some vaccines currently in development.

I. Zika Virus in Florida

While ZIKV was first discovered in 1947 in Uganda, the first human cases were not reported until 1952. Since then, ZIKV has spread to other parts of Africa, Asia, the Pacific Islands, and the Americas. In December 2015, the first cases of ZIKV infection in Florida were identified in people who traveled to ZIKV-affected countries. From 2016–2017, there were 1,347 travel-associated cases reported in Florida (1,122 cases in 2016 and 225 in 2017). The first locally acquired case was identified in Miami-Dade County in July 2016. Multiple introductions in 2016 resulted in 300 reported local cases in the state, with many linked to four areas of active transmission in Miami-Dade County. While many of these cases were exposed in Miami-Dade County, sporadic local introductions also occurred in 2016 in Broward, Palm Beach, and Pinellas counties. In 2017, two locally acquired cases were reported in Miami-Dade and Manatee counties.

Cases were also investigated for possible sexual transmission. In 2016, three sexual transmission cases were reported in Lee, Pinellas, and Polk counties. In 2017, three additional sexual transmission cases were identified in Hillsborough, Miami-Dade, and Pinellas counties. All six cases involved male to female transmission. Florida has also identified two cases of GBS related to ZIKV infection; both cases were identified in 2016. Since 2016, ten congenital Zika virus cases (eight disease cases and two infections) have been identified in Florida. Three fetal losses with Zika-related birth defects (two Zika PCR positive and one IgM positive through cerebral spinal fluid [CSF]) have also been identified. All Zika positive fetal losses were in 2016.

3. CASE DEFINITIONS

Zika Virus Disease and Infection, Non-Congenital

A. Clinical description

A person with one or more of the following not explained by another etiology:

- Clinically compatible illness that includes one or more of the following:
 - Fever (measured or reported), **or**
 - Rash, **or**
 - Arthralgia, **or**
 - Conjunctivitis;
- **Or** complication of pregnancy including one of the following:
 - Fetal loss **or**
 - Fetus or neonate with congenital microcephaly, congenital intracranial calcifications, other structural brain or eye abnormalities, or other congenital central nervous system-related abnormalities including defects such as clubfoot or multiple joint contractures;
- **Or** GBS meeting Brighton Collaboration level 1, 2, or 3 or other neurologic manifestations.

B. Laboratory criteria for diagnosis

Confirmatory:

For all locally acquired cases (including index, sporadic, and locally acquired via mosquito exposure after epidemiologic linkage or transmission in an area has been established), either of the following:

- Detection of ZIKV by culture, viral antigen, or viral RNA in serum, CSF, tissue, or other specimen (e.g., urine, whole blood, amniotic fluid, semen) by a state public health laboratory (PHL) or the Centers for Disease Control and Prevention (CDC)
- **Or** all of the following:
 - Positive enzyme immunoassay (EIA), microsphere immunofluorescence assay (MIA), or immunofluorescent assay (IF) for ZIKV IgM antibodies in serum or CSF by a PHL or CDC, **and**
 - Positive neutralizing antibody titers by plaque reduction neutralization test (PRNT) against ZIKV by a PHL or CDC, **and**
 - Negative neutralizing antibody titers by PRNT against dengue virus (DENV) (or other flaviviruses endemic to the region where exposure occurred) by a PHL or CDC.

For imported cases, either of the following:

- Detection of ZIKV by culture, viral antigen, or viral RNA in serum, CSF, tissue, or other specimen (e.g., urine, whole blood, amniotic fluid, semen)
- **Or** all of the following:
 - Positive EIA, MIA, or IF for ZIKV IgM antibodies in serum or CSF; **and**
 - Positive neutralizing antibody titers by PRNT against ZIKV; **and**
 - Negative neutralizing antibody titers by PRNT against DENV (or other flaviviruses endemic to the region where exposure occurred).

Presumptive:***For index or sporadic cases acquired locally via mosquito exposure, all of the following:***

- Positive EIA, MIA, or IF for ZIKV IgM antibodies in serum or CSF by a PHL or CDC; **and**
- Positive neutralizing antibody titers by PRNT against ZIKV by a PHL or CDC; **and**
- Absence of positive EIA, MIA, or IF for DENV IgM antibodies (or other flaviviruses endemic to the region where the exposure occurred).

For cases acquired locally via mosquito exposure after epidemiologic linkage or transmission in an area has been established, one or more of the following:

- All of the following:
 - Positive or equivocal EIA, MIA, or IF for ZIKV IgM antibodies in serum or CSF by a PHL or CDC; **and**
 - Positive neutralizing antibody titers by PRNT against ZIKV by a PHL or CDC; **and**
 - Absence of positive EIA, MIA, or IF for DENV IgM antibodies (or other flaviviruses endemic to the region where the exposure occurred).
- **Or** all of the following:
 - Positive EIA, MIA, or IF for ZIKV IgM antibodies in serum or CSF by a PHL or CDC; **and**
 - Negative or equivocal EIA, MIA, or IF for DENV IgM antibodies (or other flaviviruses endemic to the region where the exposure occurred) by a PHL or CDC; **and**
 - Absence of a negative neutralizing antibody titers by PRNT against ZIKV;
- **Or** both of the following:
 - Seroconversion from negative for ZIKV IgM antibodies in an acute-phase specimen to positive for ZIKV IgM antibodies in a convalescent-phase specimen by EIA, MIA, or IF in serum or CSF by a PHL or CDC **and**
 - Negative or equivocal EIA, MIA, or IF for DENV IgM antibodies (or other flaviviruses endemic to the region where the exposure occurred) by a PHL or CDC;
- **Or** all of the following:
 - Positive EIA, MIA, or IF for ZIKV IgM antibodies in serum or CSF by a PHL or CDC; **and**
 - Negative DENV polymerase chain reaction (PCR) by a PHL or CDC; **and**
 - Negative or equivocal EIA, MIA, or IF for DENV IgM antibodies by a PHL or CDC; **and**
 - Seroconversion from negative for DENV IgG antibodies in an acute-phase specimen to positive for DENV IgG antibodies in a convalescent-phase specimen by EIA, MIA, or IF by a PHL or CDC;
- **Or** all of the following:
 - Positive EIA, MIA, or IF for ZIKV IgM antibodies in serum; **and**
 - Positive EIA, MIA, or IF for DENV IgM antibodies (or other flaviviruses endemic to the region where the exposure occurred); **and**
 - Epidemiological linkage to a confirmed or probable ZIKV case

For imported cases in pregnant women, all of the following:

- Positive or equivocal EIA, MIA, or IF for ZIKV IgM antibodies in serum or CSF; **and**
- Positive neutralizing antibody titers by PRNT against ZIKV; **and**
- Absence of positive EIA, MIA, or IF for DENV IgM antibodies (or other flaviviruses endemic to the region where the exposure occurred).

For non-pregnant, imported cases, one or more of the following:

- All of the following:
 - Positive or equivocal EIA, MIA, or IF for ZIKV IgM antibodies in serum or CSF; **and**
 - Positive neutralizing antibody titers by PRNT against ZIKV; **and**
 - Absence of positive EIA, MIA, or IF for DENV IgM antibodies (or other flaviviruses endemic to the region where the exposure occurred);
- **Or** both of the following:
 - Seroconversion from negative for ZIKV IgM antibodies in an acute-phase specimen to positive for ZIKV IgM antibodies in a convalescent-phase specimen by EIA, MIA, or IF in serum or CSF **and**
 - Negative or equivocal EIA, MIA, or IF for DENV IgM antibodies (or other flaviviruses endemic to the region where the exposure occurred);
- **Or** all of the following:
 - Positive EIA, MIA, or IF for ZIKV IgM antibodies in serum or CSF; **and**
 - Negative DENV PCR; **and**
 - Negative or equivocal EIA, MIA, or IF for DENV IgM antibodies; **and**
 - Seroconversion from negative for DENV IgG antibodies in an acute-phase specimen to positive for DENV IgG antibodies in a convalescent-phase specimen by EIA, MIA, or IF;
- **Or** all of the following:
 - Positive EIA, MIA, or IF for ZIKV IgM antibodies in serum or CSF; **and**
 - Negative DENV PCR; **and**
 - Negative or equivocal EIA, MIA, or IF for DENV IgM antibodies; **and**
 - Positive for DENV IgG antibodies;
- **Or** all of the following:
 - Positive EIA, MIA, or IF for ZIKV IgM antibodies in serum; **and**
 - Positive EIA, MIA, or IF for DENV IgM antibodies (or other flaviviruses endemic to the region where the exposure occurred); **and**
 - Epidemiological linkage to a confirmed or probable ZIKV case.

Supportive:***For locally acquired cases and imported cases in pregnant women, one or more of the following:***

- Both of the following:
 - Positive ZIKV PCR by a commercial laboratory **and**
 - No testing performed by a PHL or CDC on the same specimen

- **Or** both of the following:
 - Positive EIA, MIA, or IF for ZIKV IgM antibodies by a commercial laboratory **and**
 - No testing performed by a PHL or CDC on the same specimen.

For all cases, one or more of the following:

- All of the following:
 - Positive, equivocal, or indeterminate EIA, MIA, or IF for ZIKV IgM antibodies in serum or CSF; **and**
 - Absence of positive DENV IgM antibodies (or other flaviviruses endemic to the region where the exposure occurred); **and**
 - Absence of positive or negative neutralizing antibody titers by PRNT against ZIKV;
- **Or** both of the following:
 - Positive neutralizing antibody titers by PRNT against ZIKV **and**
 - Absence of positive DENV IgM antibodies (or other flaviviruses endemic to the region where the exposure occurred);
- **Or** all of the following:
 - Positive ZIKV PCR by a commercial laboratory; **and**
 - Negative or equivocal ZIKV PCR by a PHL or CDC for the same specimen; **and**
 - Absence of a positive or equivocal EIA, MIA, or IF for ZIKV IgM antibodies by a PHL or CDC for the same specimen; **and**
 - No additional specimens collected;
- **Or** all of the following:
 - Positive or equivocal EIA, MIA, or IF for ZIKV IgM antibodies by a commercial laboratory; **and**
 - Negative EIA, MIA, or IF for ZIKV IgM antibodies by a PHL or CDC for the same specimen; **and**
 - Positive neutralizing antibody titers by PRNT against ZIKV.

Not a case:

One or more of the following:

- Both of the following:
 - Positive or equivocal EIA, MIA, or IF for ZIKV IgM antibodies by a commercial laboratory **and**
 - Negative EIA, MIA, or IF for ZIKV IgM antibodies by a PHL or CDC for the same specimen;
- **Or** all of the following:
 - Positive ZIKV PCR by a commercial laboratory; **and**
 - Negative or equivocal ZIKV PCR by a PHL or CDC; **and**
 - Negative or indeterminate EIA, MIA, or IF for ZIKV IgM antibodies by a PHL or CDC for the same specimen; **and**
 - Negative or indeterminate EIA, MIA, or IF for ZIKV IgM antibodies in a convalescent specimen collected 7 days to 12 weeks after the first specimen;
- **Or** negative ZIKV PCR;

- Or negative EIA, MIA, or IF for ZIKV IgM antibodies;
- Or testing is otherwise determined to be falsely positive by case reviewer.

C. Epidemiologic Linkage Criteria

General epidemiological criteria:

One or more of the following:

- Resides in or recent travel to an area with known ZIKV transmission, **or**
- Receipt of blood or blood products within 30 days of symptom onset, **or**
- Receipt of organ or tissue transplant within 30 days of symptom onset, **or**
- Epidemiologically linked to a confirmed or probable case, **or**
- Sexual contact with a confirmed or probable case of ZIKV infection or person with recent travel to an area with known ZIKV transmission, **or**
- Likely vector exposure in an area with suitable seasonal and ecological conditions for potential local vector-borne transmission.

Confirmatory perinatal linkage:

A mother whose baby is a confirmed congenital Zika case.

Presumptive perinatal linkage:

A mother whose baby is a probable congenital Zika case.

Supportive perinatal linkage:

A mother whose baby is a suspect congenital Zika case.

D. Case classification

Zika virus disease

Confirmed:

Either of the following:

- A clinically compatible illness in a person with confirmatory laboratory evidence and general epidemiological criteria **or**
- A clinically compatible illness in a mother with confirmatory perinatal linkage.

Probable:

Either of the following:

- A clinically compatible illness in a person with presumptive laboratory evidence and general epidemiological criteria **or**
- A clinically compatible illness in a mother with presumptive perinatal linkage.

Suspect:

Either of the following:

- A clinically compatible illness in a person with supportive laboratory evidence and general epidemiological criteria **or**
- A clinically compatible illness in a mother with supportive perinatal linkage.

Zika virus infectionConfirmed:

Either of the following:

- A person with confirmatory laboratory evidence with general epidemiological criteria **or**
- A mother with confirmatory perinatal linkage.

Probable:

Either of the following:

- A person with presumptive laboratory evidence with general epidemiological criteria **or**
- A mother with presumptive perinatal linkage.

Suspect:

Either of the following:

- A person with supportive laboratory evidence with general epidemiological criteria **or**
- A mother with supportive perinatal linkage.

Comment

Cross-reaction with related flaviviruses (e.g., dengue, West Nile, yellow fever, Japanese encephalitis viruses) on serological tests is common, and results may be difficult to interpret. Due to this cross-reactivity, it is important to ask if there has been any lifetime travel to a flavivirus-endemic country or vaccination for yellow fever or Japanese encephalitis viruses. In addition, people with dengue infection often test positive for ZIKV IgM.

Due to the cross-reactivity seen among flaviviruses, individuals testing positive for both ZIKV and DENV IgM should be reported as flavivirus disease and infection (Merlin disease code=07000). PRNT is not required to meet these criteria; however, if a PRNT is performed, there should be positive neutralizing antibody titers to both ZIKV and DENV. If an individual with flavivirus results is epidemiologically linked to a confirmed or probable ZIKV or DENV case, the case should not be reported as a flavivirus case.

Clinicians should also consider testing for dengue and chikungunya fever for suspect cases of ZIKV disease if fever was reported. As testing capacity allows, all specimens meeting the requirements for ZIKV disease PCR testing at the Bureau of Public Health Laboratories (BPHL) will also be tested for dengue and chikungunya viruses if the patient reported fever. All specimens collected in the first four days of illness and meeting standard requirements for dengue and chikungunya testing will also be tested for Zika virus by PCR if travel to a ZIKV disease endemic area is reported.

Differentiating between ZIKV and DENV infections in PCR-negative patients with positive flavivirus labs

- Conjunctivitis and pruritic rash are more common with ZIKV disease than dengue fever.
- Thrombocytopenia and leukopenia are more common and severe in cases of dengue fever compared to ZIKV disease.
- ZIKV is not known to cause severe syndromes that can be seen with DENV (dengue hemorrhagic fever or dengue shock syndrome).

- ZIKV IgM titers are usually positive in dengue fever patients. DENV IgM titers may or may not be positive in ZIKV disease patients. EIA IgM results from BPHL are not quantitative, and the values derived from this assay cannot be compared between illnesses.
- For non-PCR positive cases, dengue fever cases should be created instead of ZIKV disease cases if one of the following is true:
 - The clinician ordered dengue testing and did not request Zika testing, and dengue IgM was positive; or
 - The clinician ordered Zika testing and Zika IgM was negative, while dengue IgM was positive; or
 - PRNT testing is positive for dengue and negative for Zika.

Acute and convalescent specimens from people with infections believed to be Florida-acquired should be sent to BPHL. Acute specimens from people with infections believed to be acquired outside Florida do not need to be forwarded to BPHL unless the specimen is from a pregnant woman, infant, or possible GBS case.

Zika Virus Disease and Infection, Congenital

A. Clinical description

Liveborn infant with congenital microcephaly, intracranial calcifications, structural brain or eye abnormalities, or other congenital CNS-related abnormalities not explained by another etiology.

B. Laboratory criteria for diagnosis

Confirmatory:

Either of the following:

- Detection of ZIKV by culture, viral antigen, or viral RNA in fetal tissue, amniotic fluid, neonatal serum, cerebrospinal fluid (CSF), urine, or umbilical cord blood* performed by a state public health laboratory (PHL) or the Centers for Disease Control and Prevention (CDC) in a specimen collected within 2 days of birth (or later if perinatal infection has been ruled out)

- **Or** all of the following:
 - Positive enzyme immunosorbent assay (EIA) or immunofluorescent assay (IFA) test for ZIKV IgM antibodies in neonatal serum, CSF, or umbilical cord blood* collected within 2 days of birth (or later if perinatal infection has been ruled out);
 - **And** positive neutralizing antibody titers by plaque reduction neutralization test (PRNT) against ZIKV;
 - **And** Negative neutralizing antibody titers by PRNT against dengue virus (DENV) or other flaviviruses endemic to the region where exposure occurred.

Presumptive:

- Both of the following:
 - Positive or equivocal EIA or IFA test for ZIKV IgM antibodies in neonatal serum, CSF, or umbilical cord blood* collected within 2 days of birth (or later if perinatal infection has been ruled out)
 - **And** Positive neutralizing antibody titers by PRNT against ZIKV;

- **Or** all of the following:
 - Positive or equivocal EIA or IFA test for ZIKV IgM antibodies in neonatal serum, CSF, or umbilical cord blood* collected within 2 days of birth or later if perinatal infection has been ruled out;
 - **And** negative or equivocal EIA or IFA test for DENV IgM antibodies or IgM antibodies to other flaviviruses endemic to the region where the exposure occurred;
 - **And** No PRNT performed.

Supportive:

- All of the following:
 - Positive or equivocal EIA or IFA test for ZIKV IgM antibodies in neonatal serum, CSF, or umbilical cord blood;*
 - **And** no DENV IgM testing performed;

- **And** no PRNT performed;
- **And** mother's test results do not rule out recent ZIKV infection;

- **Or** positive neutralizing antibody titers by PRNT against ZIKV in a sample collected ≥ 18 months after birth;

- **Or** both of the following:
 - Positive EIA or IFA for ZIKV IgM antibodies from a commercial laboratory;
 - **And** no testing performed by a PHL or CDC on the same specimen;

- **Or** both of the following:
 - Positive ZIKV PCR by a commercial laboratory;
 - **And** no testing performed by a PHL or CDC on the same specimen.

Not a case:

One or more of the following:

- Both of the following:
 - Positive or equivocal EIA or IFA for ZIKV IgM antibodies by a commercial laboratory;
 - **And** negative or indeterminate EIA or IFA for ZIKV IgM antibodies by a PHL or CDC for the same specimen;

- **Or** all of the following:
 - Positive ZIKV PCR by a commercial laboratory;
 - **And** negative or equivocal PCR by a PHL or CDC;
 - **And** absence of a positive or equivocal EIA or IFA for ZIKV IgM antibodies by a PHL or CDC for the same specimen;

- **Or** positive neutralizing antibody titers by PRNT against ZIKV in a sample collected < 18 months after birth;

- **Or** negative neutralizing antibody titers by PRNT against ZIKV;

- **Or** negative ZIKV PCR;

- **Or** negative or indeterminate EIA or IFA for ZIKV IgM antibodies;

- **Or** testing is otherwise determined to be falsely positive by case reviewer.

*Note: while collection of umbilical cord blood was initially recommended, neonatal serum is the preferred specimen type. Umbilical cord blood should only be submitted for testing when no serum is available.

C. Epidemiologic Linkage Criteria

Mother

One or more of the following:

- Resides in or recent travel to an area with known ZIKV transmission

- **Or** sexual contact with a confirmed or probable case of ZIKV infection or person with recent travel to an area with known ZIKV transmission,
- **Or** receipt of blood or blood products within 30 days of symptom onset,
- **Or** receipt of organ or tissue transplant within 30 days of symptom onset,
- **Or** association in time and place with a confirmed or probable case,
- **Or** likely vector exposure in an area with suitable seasonal and ecological conditions for potential local vector-borne transmission.

Infant

Both of the following:

- Mother meets the epidemiological criteria
- **And** infant meets either of the following:
 - No travel to an area with known ZIKV transmission reported for the infant since birth if tested after 18 months
 - **Or** after reviewing postnatal travel history and 18-month test results, case reviewer determines low ZIKV exposure risk postnatally.

D. Case classification**Zika virus disease**Confirmed:

- Clinically compatible congenital disease in a neonate with confirmatory laboratory evidence whose mother meets the epidemiologic criteria.

Probable:

- Clinically compatible congenital disease in a neonate with presumptive laboratory evidence whose mother meets the epidemiologic criteria.

Suspect:

Either of the following:

- Clinically compatible congenital disease in a neonate with supportive laboratory evidence whose mother meets the epidemiologic criteria
- **Or** clinically compatible congenital disease in an infant with supportive laboratory evidence and the infant epidemiologic criteria.

Zika virus infectionConfirmed:

- A neonate with confirmatory laboratory evidence whose mother meets the epidemiologic criteria.

Probable:

- A neonate with presumptive laboratory evidence whose mother meets the epidemiologic criteria.

Suspect:

Either of the following:

- A neonate with supportive laboratory evidence whose mother meets the epidemiologic criteria
- **Or** an infant with supportive laboratory evidence and the infant epidemiologic criteria.

Comment

As part of the complete evaluation of congenital microcephaly or other CNS birth defects, testing for other congenital infections such as syphilis, toxoplasmosis, rubella, cytomegalovirus infection, lymphocytic choriomeningitis virus infection, and herpes simplex virus infections should be considered. An assessment of potential genetic and other teratogenic causes of the congenital anomalies should also be performed.

Cross-reaction with related flaviviruses (e.g., dengue, West Nile, yellow fever, Japanese encephalitis viruses) on serological tests is common and results may be difficult to interpret. Due to this cross-reactivity, it is important to ask if there has been any lifetime travel by the mother to a flavivirus-endemic country or vaccination for yellow fever or Japanese encephalitis viruses. In addition, people with dengue infection often test positive for ZIKV IgM. It is also important to get the lifetime travel history for the infant, particularly if PRNT testing is going to be performed at 18 months of age to assess congenital exposure.

Specimens from infants with possible congenital infections should be sent to the Bureau of Public Health Laboratories.

4. LABORATORY TESTING

A. Criteria for diagnosis

Confirming the diagnosis of ZIKV infection can be made using a variety of testing methods. BPHL provides confirmatory laboratory testing services for specimens forwarded for confirmation that meet epidemiological criteria (appropriate signs and symptoms as well as travel history).

1. ZIKV PCR positive commercial laboratory test results should be confirmed by BPHL for pregnant women, and suspected local, congenital, and GBS cases. Specimens testing positive, equivocal, or inconclusive/indeterminate for IgM antibodies should be forwarded to BPHL for repeat IgM and plaque reduction neutralization testing (PRNT), as needed. If available, specimens for non-pregnant, symptomatic travel-associated cases and Zika reactive blood donors may also be forwarded for confirmation.
2. Health care providers should collect both serum and urine specimens from patients who are symptomatic. Uninsured symptomatic individuals with two of the key symptoms reported (fever, rash, arthralgia, and conjunctivitis) or GBS and travel to an area experiencing ZIKV activity can be sent to BPHL. Specimens from individuals without travel with three of the key symptoms should be sent to BPHL.
3. Serum and urine may also be submitted for uninsured pregnant women with appropriate epidemiologic risk factors, regardless of reported symptoms. Specimens from all infants born to women who may have been exposed to ZIKV during pregnancy should also be sent. Please see below for more information on testing of pregnant women, infants, and other specimen types that may be collected for PUIs.

4. There is some cross-reactivity between ZIKV antibody and other closely related flavivirus antibodies on serologic tests, and it is important for dengue testing or testing for other appropriate flaviviruses to also be conducted. Please reference the information at the end of the Zika [case definition](#) to help interpret laboratory results. In addition, the following link may be useful for the interpretation of serological test results:
www.cdc.gov/mmwr/volumes/65/wr/mm6521e1.htm.
In cases where a person is not PCR-positive but tests positive for both Zika and dengue IgM, please review the flavivirus case definition at the following link:
www.floridahealth.gov/gsi.

B. Services available at the Bureau of Public Health Laboratories (BPHL)

BPHL can test clinical specimens for Zika by viral isolation/PCR or antibody detection by EIA and PRNT.

C. Testing requests

1. Submitting specimens/isolates to BPHL
 - a. **Testing requires prior approval by CHD epidemiology staff before specimen submission.**
 - b. BPHL staff should be notified of specimen submission and all submissions should be accompanied by the Clinical Lab Submission Form found at www.floridahealth.gov/diseases-and-conditions/disease-reporting-and-management/disease-reporting-and-surveillance/surveillance-and-investigation-guidance/_documents/dh1847clinicallabsubmissionform.pdf
 - c. PCR testing for Zika virus at BPHL can be ordered using BPHL form DH1847. Select test code 1680 (Arbovirus PCR) under the virology section and write “Zika PCR” in the comment box. IgM serologic tests can be ordered using test code 1500, Arbovirus IgM Antibody.
 - d. Include symptom onset, specimen collection date, pregnancy status (female patients), travel country/territory and dates, and whether fever was reported on all orders.
2. Specimen Collection
 - a. Specimens for PCR testing should be collected within the first 14 days of illness or exposure: serum (2 mL serum/red or red/grey marble tiger top tube) AND urine specimen (1–2 mL, 5 mL max, in a sterile tube) should both be collected. Do not submit urine in urine collection cups as these tend to leak during transport. Use a sterile tube with secure closure to prevent leakage. Each tube should be in a separate zip-seal bag. Use sterile tubes for urine and seal or cap container(s) securely to avoid leakage and loss of specimen. Other specimen types such as whole blood (EDTA), CSF, and amniotic fluid may also be appropriate in consultation with the Arbovirus Surveillance Coordinator. These specimens should be submitted with a patient-matched serum specimen.
 - b. Serum only for IgM testing is preferred if collected ≥ 14 days following symptom onset or exposure. **PCR testing will only be performed if serological tests are positive and the patient is pregnant or has no travel.**
 - c. Specimens (serum, whole blood, and CSF only) meeting the requirements for Zika PCR testing at BPHL will also be tested for dengue and chikungunya via trioplex

- PCR. Dengue IgM testing will be performed if Zika IgM testing is positive. If a patient reports fever and dengue or chikungunya IgM testing is also desired, make sure to request that testing as well on the Clinical Lab Submission Form. The laboratory may not automatically reflex to that testing if not requested.
- d. **FOR PREGNANT WOMEN ONLY:** serum and urine specimens for PCR and IgM testing are preferred regardless of time of collection following symptom onset or exposure.
 - i. The most current CDC guidelines for testing of pregnant women are available at www.cdc.gov/mmwr/zika_reports.html. Due to the declining prevalence of ZIKV in the Americas and the possibility of prolonged IgM antibody detection, the following two groups should be tested at BPHL: uninsured symptomatic pregnant women and uninsured asymptomatic pregnant women with ongoing possible ZIKV exposure.
 - ii. Testing at BPHL should not be approved for pregnant women without risk factors for ZIKV exposure (e.g., no travel history to areas with active transmission, no sexual contact with a partner who lives in or traveled to an area with active ZIKV infection, etc.) unless the woman meets local PUI criteria. These individuals should be counseled on the risks and benefits of testing (e.g., false positives and negatives) and reassured that testing is unnecessary. Specimens should go directly to the commercial laboratory if the woman has a private provider and insurance.
 - iii. Unless a Zika-associated abnormality is reported in the infant, testing of pregnant women with possible ZIKV exposure should occur during pregnancy through the end of the first postnatal week. **For testing and investigation purposes, a woman is considered pregnant if her possible ZIKV exposure occurred at any point during pregnancy or within two months of conception.** The length of ZIKV IgM detection is unknown, but epidemiological and laboratory data indicate that it may persist for more than 12 weeks. Specimens collected after 12 weeks can still be tested; however, a negative IgM result does not definitively prove that the person wasn't exposed to Zika virus as testing may not occur soon enough for Zika IgM antibodies to still be detectable.
 - iv. **If a pregnant woman tests positive for Zika by PCR and she and her provider are interested in additional testing,** coordinate with the physician to collect specimens weekly until PCR results are negative. Some research has speculated that viral replication in the placenta or fetus could be a cause of prolonged viremia. However, it is unknown how prolonged viremia can impact the fetus during pregnancy.
 - e. **FOR INFANTS ONLY:** Serum should be collected from infants whose mother had travel to a country or region with ZIKV transmission at any point in pregnancy or the two months before conception or who had possible sexual exposure with a male partner that traveled in the 3 months prior to sexual intercourse. Infant testing should be expedited if the mother has not previously been tested. Specimens can be collected from both mother and infant at birth if not previously tested.
 - i. **Infant specimens should be collected within two days of birth.** Please submit the requested volumes indicated above; if this is not possible a minimum

- of 0.5 mL for each specimen type may be submitted. It is important for specimens to be clearly marked as belonging to the infant vs. the mother. If specimens are not collected within two days of birth, attempt to collect specimens at the next doctor's visit. CSF may also be collected if the infant has an abnormality and the CSF is being collected for other purposes. For infants with abnormalities consistent with congenital Zika syndrome (CZS) and potential ZIKV exposure during pregnancy, serum specimens can also be submitted once the infant is 18 months old. PRNT testing will be performed on these specimens to determine whether there was a congenital exposure for the infant. Testing will be available for infants up to 36 months of age. Please reach out to the Arbovirus Surveillance Coordinator or Pregnancy Registry Coordinator for testing approval.
- ii. Amniotic fluid: Based on experience with other congenital infections, amniocentesis has been used in the past to diagnose intrauterine infections. However, the performance of PCR testing of amniotic fluid for Zika virus infection has not been evaluated. Furthermore, the risk for microcephaly or other anomalies when Zika virus RNA is detected in amniotic fluid is not known. If amniotic fluid is being collected for other purposes, such as genetic testing, ZIKV PCR may be performed after approval by the Arbovirus Surveillance Coordinator or Pregnancy Registry Coordinator and if a patient-matched serum specimen is also submitted.
 - iii. Pathology specimens, such as formalin fixed placental and other tissues following a birth or fetal/infant loss, may also be collected in a sterile container and sent to BPHL on a case-by-case basis. These specimens will be referred to the CDC's Infectious Disease Pathology Branch for testing as appropriate. Refer to the CDC Collection and Submission of Fetal Tissues for Zika Virus Testing guidance document at www.cdc.gov/zika/laboratories/test-specimens-tissues.html for further details.
3. Packaging and shipping
- a. Specimens can be sent to the assigned BPHL (Jacksonville, Tampa, or Miami [PCR only]) for testing.
 - b. Specimen labeling
 - i. If the specimen is acute (collected 14 or fewer days post onset), the serum or urine should be shipped frozen on dry ice in an insulated cooler. Hold specimens in an insulated container with dry ice or an ultra-low freezer until shipped. This is best for virus isolation, but viral RNA may still be detectable in freshly collected acute serum or urine that is immediately sent overnight to the laboratory with frozen gel ice in an insulated cooler.
 - ii. If the specimen is convalescent (collected 15 or more days post onset), the serum may be shipped frozen on dry ice or cold with frozen gel ice in an insulated cooler because the serum will be tested for antibody only. Hold serum in a refrigerator until shipped.
 - iii. Serum is stored in standard sterile airtight tubes or a serum separator tube (separated prior to refrigeration and shipping) without added media or fixative.
 - iv. Each specimen must be labeled with the patient's name, date of birth, and date of collection.
 - c. A DOH Clinical Laboratory Submission Form must be included for each patient, listing all specimens. Follow packaging and shipping guidelines for diagnostic

- specimens (Biological Substance, Category B, UN3373). All suspect diagnostic specimens must be shipped and packaged according to International Air Transport Association (IATA) and Department of Transportation (DOT) Packaging Instructions 650 for Biological Substance, Category B Agents. Per these regulations anyone who handles, offers for transport, or transports specimens must be trained and certified to do so. Specifications state specimens must be packed in a basic triple packaging system consisting of a primary watertight container wrapped with absorbent material, secondary watertight container, and an outer shipping package. Enclose an itemized list of contents between the secondary packaging and the outer packaging.
- d. Contact BPHL for packaging and shipping training dates. BPHL conducts approximately 20 face-to-face trainings per year throughout Florida, free of charge. DOH employees must register for the classes in the DOH online training system TRAIN-FL. For shipping guidance, contact BPHL. Additional shipping trainings are also available commercially through vendors.
 - e. To expedite receipt of specimens at the laboratory, overnight or two-day express shipment is suggested. If sera are shipped on Friday, the package must be clearly marked for "Saturday Morning Delivery."
4. Contact BPHL-Tampa, Jacksonville, or Miami with questions:
www.floridahealth.gov/programs-and-services/public-health-laboratories/locations/index.html.

D. Interpretation of results

Individuals with only an IgM positive result will require additional testing. If pregnant, a suspected local case, congenital, or Guillain-Barré syndrome case, PRNT testing may be required before a complete interpretation can be made. Testing at BPHL, another state public health lab, or at CDC is required for these individuals. For non-pregnant imported cases, dengue serology testing may aid in result interpretation. The following link may be useful for the interpretation of serological test results: www.cdc.gov/mmwr/volumes/65/wr/mm6521e1.htm. If a PCR-negative person tests positive for both Zika IgM and dengue IgM, please review the flavivirus case definition at the following link: www.floridahealth.gov/diseases-and-conditions/disease-reporting-and-management/disease-reporting-and-surveillance/surveillance-and-investigation-guidance/index.html#W.

For any questions about lab results from BPHL or other labs, consult the Arbovirus Surveillance Coordinator or BPHL-Tampa or Jacksonville. Interpretation of each of the tests is dependent upon the time of specimen collection relative to the date of symptom onset, the patient's previous arbovirus infection history, and serum cross-reactivity within the flavivirus antigenic complex. In Florida, previous WNV or DENV infection, previous yellow fever, dengue, or Japanese encephalitis vaccination are the most common factors that can complicate the interpretation of antibody tests. In addition, current infections with herpes simplex virus, Epstein-Barr virus, *Streptococcus*, influenza, or other pathogens may also complicate the interpretation of antibody tests as cross reactivity has been identified between other flaviviruses and these pathogens.

5. CASE INVESTIGATION**A. Contact the physician or hospital**

1. **Florida Confidential Vector-Borne Disease Case Report form (required):** This form can be used to guide the interview and can be completed during the interview. www.floridahealth.gov/diseases-and-conditions/disease-reporting-and-management/disease-reporting-and-surveillance/_documents/crf-zika-confidential.pdf.
2. Verify if a Zika illness has been diagnosed in the reported case.
3. Obtain the following:
 - a. Date of onset
 - b. Signs and symptoms
 - c. Travel history
 - d. Pregnancy status and related questions
 - e. Any similar illness in other contacts
 - f. Predisposing conditions (e.g., immunosuppression)
 - g. Tests performed (including EIA, PCR, culture, or any other test performed)
 - h. Treatment for pre-existing conditions (e.g., rheumatic arthritis)
4. Ask what information has been given to the patient, including whether the patient knows about the diagnosis and risk factors.
5. Ask if patients were advised to avoid mosquito bites while ill and to take sexual precautions.
6. Obtain as much demographic information as possible, including contact information (home, cellular, pager, and work numbers). Ask how and where the patient can be contacted (i.e., at hospital or home).
7. Notify the physician that you will be contacting the case as DOH follows up on all cases of ZIKV infection to assess risks factors, to better characterize the occurrence of these infections in Florida, and to identify potential means for preventing further transmission. It is also appropriate at this point to determine if the physician has any concerns about the health department contacting the case.
8. Request medical records for any PUIs that have inpatient hospitalizations potentially linked to ZIKV infection. Medical records should also be requested for pregnant cases and possible CZS cases. See [Section 6](#) for more information on suggested medical record collection for infants and pregnant women.
9. The CHD designee will arrange acute and convalescent blood specimen collection and submission to BPHL, as appropriate, to confirm infection with a vector-borne disease. Specimens from suspect local cases, uninsured pregnant women, infants potentially exposed to ZIKV during pregnancy, suspect GBS cases, and individuals without health insurance should be sent to BPHL.
10. If the PUI meets the case definition for a confirmed, probable, or suspect case, the CHD is responsible for reporting all required information in Merlin under the appropriate disease code.

B. Inform local mosquito control personnel of suspected Zika case (if applicable)

1. For counties with a mosquito control district, notification should occur for the following:
 - a. A symptomatic PUI that was in Florida any time from two days prior to symptom onset to 10 days post symptom onset. If reporting occurs more than two months after

- symptom onset, no notification to mosquito control is needed. Discuss this time frame with mosquito control to ensure they have no concerns.
- b. An asymptomatic PUI that has any PCR-positive lab results from a commercial or reference laboratory. If the PUI tests negative for ZIKV after confirmatory testing at BPHL, please let mosquito control know they can cease abatement efforts.
2. For counties without a mosquito control district, the County Health Officer should alert the state epidemiology office to coordinate with the Florida Department of Agriculture and Consumer Services (FDACS) regional response team if either a or b below are met. FDACS team deployment will be determined on a case-by-case basis depending on the risk for sustained local ZIKV transmission.
 - a. A symptomatic person that meets PUI criteria with PCR-positive laboratory results from a commercial or reference laboratory.
 - b. An asymptomatic PUI with PCR-positive lab results from a commercial or reference laboratory. If the PUI tests negative for ZIKV after confirmatory testing at BPHL, please let the FDACS regional response team know they can cease abatement efforts.
 3. Provide work or other addresses as appropriate to mosquito control for PUIs that have a high risk for mosquito exposure due to occupation or other activities (i.e., primarily outdoors).

C. Interview the case

1. Contact the case or the case's proxy to complete an interview as soon as possible after being reported to optimize recall.
 - a. Make at least three phone call attempts to reach the case. Additional tools such as LexisNexis can help to potentially identify additional ways to contact the person. If a CHD does not have access to LexisNexis and needs assistance with looking up additional contact information, please reach out to the Arbovirus Surveillance Coordinator.
 - b. Calls should be made at different times of the day, with at least one attempt in the evening.
 - c. If unable to reach by phone or certified letter, a field visit to the home should be made for suspected locally acquired cases.
 - d. Additional methods for tracking down pregnant women/infants for follow-up:
 - i. Health care providers
 - ii. Florida SHOTS
 - iii. Contact the Pregnancy Registry Coordinator or Arbovirus Surveillance Coordinator to query vital statistics data for birth information
 - iv. Contact the local WIC program
2. **Florida Confidential Vector-Borne Disease Case Report form (required):**
This form can be used to guide the interview and can be completed during the interview. www.floridahealth.gov/diseases-and-conditions/disease-reporting-and-management/disease-reporting-and-surveillance/_documents/crf-zika-confidential.pdf.
3. Items to cover during interview include:
 - a. Provide brief background on disease, including mode of transmission, incubation period, symptoms, etc.
 - b. Remind patient to avoid mosquito bites while ill.

- c. Confirm symptoms and onset dates. The desired time frame for the questions below, unless otherwise stated, is two weeks prior to symptom onset, within the last two years for asymptomatic individuals, and within the last six months for blood donors.
- d. Ask for travel and activity history
 - i. Travel history outside county of residence, state, or country including dates.
 - ii. Travel to Zika endemic areas and ask if additional travelers were ill (all travelers should be advised to use mosquito bite precautions for 3 weeks post-travel). A detailed travel history, including home, work, and other locations at risk for mosquito bite exposure should be collected for suspect locally acquired cases. Zika endemic areas can be found at www.cdc.gov/zika/geo/index.html
 - iii. Sexual activities with a partner who **traveled to or lived in** an area endemic to Zika. Please note that this question may be confusing for some individuals, particularly among people who have moved from another country to Florida. If they had unprotected sex during the exposure period in that country or after arriving in Florida (eight weeks for female partners and three months for male partners) then the answer to this question is most likely yes.
 - iv. Any febrile illnesses or travel reported for household members or other contacts in the month prior to patient's onset.
 - v. Occupation and address
 - vi. Hobbies (gardening, fresh water fishing, hunting) and locations
 - vii. Other outdoor activities (smoking outside, etc.) and locations
 - viii. Use of preventive measures (intact screens, regular use of repellents, drain standing water, etc.)
- e. Obtain pregnancy information, if applicable. For surveillance purposes, a woman is considered pregnant if potentially exposed to ZIKV during pregnancy or within the two months prior to pregnancy, not necessarily if she is pregnant at the time of testing. This may be difficult to determine for pregnant women living in endemic areas.
- f. Collect history of blood transfusions, organ transplants, or blood donations in the past six months.
- g. As part of the interview, provide basic education to the cases about personal protection measures to prevent mosquito bites and the "Drain and Cover" message. Emphasize the need to drain standing water at least once a week.
- h. Provide basic education on the risk of sexual transmission and the importance of taking appropriate precautions.
- i. Arrange for additional specimens to be collected, if needed.

D. Merlin data entry

1. Create a case in Merlin under the appropriate disease code. Cases should be created for both imported and locally acquired cases. Individuals meeting case criteria should have the case survey selected on the basic case screen in Merlin. **Cases should also be created for non-Florida residents who were exposed or tested in Florida.** In addition, cases should be created for PUIs. A person who is having a specimen tested for ZIKV at BPHL or CDC is considered a PUI. Counties can choose whether to enter PUIs under the case or PUI survey on the basic case screen in Merlin. The PUI survey is a shorter survey that streamlines data entry for users and is limited to key pieces of information.

- a. Imported and locally acquired Zika cases should be reported in Merlin as either non-congenital (Merlin disease code=06010) or congenital (Merlin disease code=06012) based on case definition. Both symptomatic and asymptomatic individuals are included under the same disease codes.
2. Enter the data collected into Merlin, being sure to include all required fields on the Basic Data screen, complete the Case Symptoms, Travel History, and Extended Data screens, and attach all relevant medical records. If the patient was hospitalized, fill out the Health Care Visits section as well. Please associate **ALL** positive results from any laboratory and negative results from BPHL received via electronic laboratory reporting (ELR) to the case. For questions regarding lab results, please contact the Arbovirus Surveillance Coordinator.

E. Inform the Arbovirus Surveillance Coordinator on suspicion of locally acquired arbovirus infection, congenital Zika infection, or suspected Zika associated GBS.

F. Sexual case investigation

1. Ask about sexual partners' travel in the three months before symptom onset.
2. Collect serum, urine, and whole blood specimens from all partners. If transmission is suspected to be male to female, request a semen specimen as well. A disease intervention specialist may be helpful when it comes to sensitive questions or requesting specimens.
3. Collect information on dates of unprotected sex, type of sexual contact, etc., as outlined in CDC's Zika Virus Case Investigation Sexual Transmission Form:
www.cdc.gov/zika/public-health-partners/infection-case-investigation-form.docx.
4. Have mosquito control or environmental health personnel perform an evaluation of the property to help inform the decision between sexual or mosquito-borne transmission.

G. Enhanced surveillance for local cases

1. In the event of a locally acquired case of ZIKV infection, an outbreak, or an increase in the number of imported cases, alert health care providers, hospital emergency rooms, and student health centers of the potential for additional patients.
2. The Arbovirus Surveillance Coordinator will notify Florida blood banks and provide the ZIP Code(s) of likely exposure locations for a single suspect or confirmed ZIKV infection. As more detailed epidemiologic information becomes available, the ZIP Codes of concern will be adjusted accordingly. In addition, if any areas of active transmission are identified, the Department will provide those areas as well. Blood banks at a minimum will screen and defer donors as described in the OneBlood Strategy to Protect the Blood Supply From Mosquito-Borne (Arbovirus) Disease, which is available in the [List of Appendices](#). All blood donations and some plasma donations in Florida are screened for ZIKV. Donors with positive results are reported back to the CHD for additional specimen collection and follow-up. The original donation specimen should be forwarded to BPHL for repeat testing. A second specimen may also need to be collected if testing on the original donation specimen is negative or inconclusive.
3. Encourage health care providers to consider Zika in any person(s) presenting with symptoms associated with this virus. Guidance for health care providers, including one pager with information for obstetricians and clinicians can be found at <https://zikafreefl.org/>. Additional CDC guidance documents for health care providers can

be found at the following links: www.cdc.gov/mmwr/zika_reports.html and www.cdc.gov/zika/hc-providers/index.html.

4. Other enhanced notification and surveillance methods can include reverse 911 calls, monitoring Florida's Electronic Surveillance System for the Early Notification of Community-based Epidemics (ESSENCE-FL), medical record review, and media outreach.
5. Active surveillance for locally acquired ZIKV cases may also be conducted.
 - a. The primary goal of active surveillance is to identify areas serving as sites of possible ZIKV exposure. This is to ensure proper response and control measures are put into place to reduce ongoing transmission. Cluster investigations should focus on both symptomatic and asymptomatic individuals for Zika. **The Vector-Borne and Zoonotic Disease Program should be consulted prior to the start of any cluster investigation as each investigation will be tailored to the specific situation.**
 - i. Household investigations may be conducted to assess household members for ZIKV infection.
 - ii. Business investigations may be conducted if there is the potential for significant outdoor exposure (not indoor office workplace), if there are known mosquito exposures at the business, or if other employees are reporting an illness compatible with ZIKV infection. Additional outdoor worksites may be identified throughout the course of an active investigation and may also be sampled in certain circumstances.
 - iii. Door-to-door urosurveys (urine collection events) may be conducted after two or more transmission events are identified. Two positive individuals from a single household and single sporadic cases generally do not meet the criteria to conduct a community urosurvey. Households within a 150-meter radius of the properties of interest will be assessed.
 - iv. Clinic urosurveys (open to the public who live or work in a specified area) may be conducted if ongoing active transmission is suspected/confirmed in an area.
 - b. Scout out the area ahead of time. It is important to know what languages are spoken by the local population. Data obtained from the Census or American Community surveys can be useful in determining what translators may be needed. If conducting door-to-door surveys, it is also important to map locations out ahead of time to get a sense of the volume and type of homes to be surveyed. The safety of volunteers as well as potential media coverage should also be taken into consideration when planning events. Volunteers should participate in media and safety trainings prior to the start of events. Local law enforcement and communities should also be notified of any events occurring in their area.
 - c. Any pregnant women identified during active surveillance should receive a Zika kit with Zika prevention/educational materials and information about Healthy Start and should be recommended to get more complete Zika testing at the CHD.
 - d. Please contact the Arbovirus Surveillance Coordinator for more information on how to conduct a cluster investigation. Additional guidance can be found in the Guide for Local Zika Investigations for Community Surveys located in the [List of Appendices](#).
7. Immediately inform the Arbovirus Surveillance Coordinator and mosquito control of additional cases that are discovered.
 - a. Areas of ongoing active transmission will be reported to the public as they are identified. Areas of active transmission or "red zones" are determined by Central

- Office staff in conjunction with the local CHD and CDC. These red zones, per CDC guidelines, are at least 1 mile wide and pregnant women are recommended to avoid the area. CDC may choose to also issue a travel advisory to these areas. The identification of two cases within 150 meters of each other (not at the same location) increases the concern for ongoing active transmission, especially if the onset dates of the cases are more than two weeks apart but less than 45 days apart. Urosurveys may be used to confirm ongoing transmission.
- b. In addition to these areas of active transmission, a Zika cautionary area or yellow zone may be defined by CDC. Specific criteria for these yellow zones to be created include having a red zone within the county or having three locally acquired cases identified within a five-mile area over a 45-day period. The yellow zone encompasses the county, city, or other similar jurisdiction with easily identifiable borders for public communication and pregnant women are recommended to consider postponing travel to these areas.
 - c. Reproductive tissue donor deferrals may also occur in these areas.
 - d. These zones will be lifted if no new cases of confirmed local transmission are identified over a period of 45 days.

6. PREGNANT WOMEN AND INFANTS

- A. Abnormalities associated with Zika virus.** The full spectrum of birth defects and congenital Zika impacts are not entirely known. The medical records should be reviewed for the following conditions that have been associated with congenital ZIKV infection.
1. Microcephaly
 - a. This is a birth condition in which an infant's head is smaller than expected compared to babies of the same sex and age. Infants with a microcephaly diagnosis as well as a head circumference less than the 3rd percentile for sex, age, and gestational age at birth meet the case definition for microcephaly www.cdc.gov/ncbddd/birthdefects/microcephaly.html.
 - b. The infant's head circumference should be measured after birth and a second head circumference is recommended before the infant is discharged. If there is a discrepancy between these two measurements, a third measurement should be performed within the first two weeks of life. The initial head circumference measurement may be influenced by molding following vaginal birth. This link allows you to calculate the head circumference percentile at birth: <http://intergrowth21.ndog.ox.ac.uk/en/ManualEntry>.
 - c. Some infants with congenital ZIKV infection who do not have microcephaly at birth may later experience slowed head growth and develop postnatal microcephaly. Head circumference measurements are recommended at follow-up pediatrician visits. The WHO standard growth charts should be used to evaluate all measurements taken after one month of age and are available at the following link: www.cdc.gov/growthcharts/who_charts.htm#The%20WHO%20Growth%20Charts.
 2. Other abnormalities associated with the congenital Zika syndrome include intracranial calcifications, neural tube defects, hearing loss, eye abnormalities, congenital skeletal muscle and joint contractures, and central nervous system dysfunction. For more information on the abnormalities reported with Zika, please visit www.cdc.gov/pregnancy/zika/testing-follow-up/zika-syndrome-birth-defects.html.

3. **Inform the Pregnancy Registry Coordinator or Arbovirus Surveillance Coordinator of any infants born with possible congenital ZIKV infection or fetal losses in women with suspected ZIKV infection.**

B. Prenatal Follow-up

1. Current guidance for health care providers caring for pregnant women with possible ZIKV exposure are available at www.cdc.gov/mmwr/zika_reports.html.
2. A woman is considered pregnant if her possible ZIKV exposure occurred at any point during pregnancy or within two months of conception. If her possible ZIKV exposure occurred more than two months prior to conception, she would not be considered a Zika-positive pregnant woman for Zika case counting purposes.
3. Due to the short window for specimen collection (within two days of birth) it is important to request that the obstetrician let the CHD know when the pregnant woman is in labor and where she will be delivering.
4. Advise pregnant women and their sexual partner to reduce the risk of sexual transmission of ZIKV by using condoms correctly and consistently or abstain from sexual intercourse for at least three months after possible Zika virus exposure.
5. Upload medical records from prenatal visits, ultrasound reports, and mother's birth records to the mother's case in Merlin.

C. Postnatal Follow-up

1. Current guidance for the diagnosis, evaluation, and management of infants with possible congenital ZIKV infection are available at www.cdc.gov/mmwr/zika_reports.html. Outpatient management recommendations are also available at www.cdc.gov/pregnancy/zika/testing-follow-up/documents/pediatric-evaluation-follow-up-tool.pdf.
 - a. U.S. Zika Pregnancy Registry: www.cdc.gov/pregnancy/zika/research/registry.html.
 - b. CDC's registry includes Zika-positive pregnant women with a birth outcome or due date prior to March 31, 2018. For infants born to confirmed/probable cases prior to March 31, 2018, the complete two-year follow-up will be needed (see below).
 - c. Coordinate with the Pregnancy Registry Coordinator and the Arbovirus Surveillance Coordinator to collect records and conduct infant testing as needed for infants born after March 31, 2018. The need for follow-up for infants born to suspect cases will also be determined on a case-by-case basis.
 - d. **Non-Florida residents and transfer cases:** Non-Florida residents should still receive follow-up until they are no longer in Florida. Continuation of follow-up may occur if a mother/infant moves to Florida from another county, state, or territory. This may include the collection of medical records or specimens after an infant or pregnant woman moves to or within Florida. Transferred cases are not added to the county's Zika case count as they were already reported by the other state or county. Inform the Pregnancy Registry Coordinator or Arbovirus Surveillance Coordinator if the mother or infant moves outside the county.
2. Ensure all infant birth and follow-up records are attached to the infant's case in Merlin.
 - a. Cases should be created for all births regardless of whether specimens are collected. Mother and infant cases should be epi-linked as perinatal. Infant lab results should be attached to the infant's case. Lab results from the placenta,

umbilical cord specimens, or amniotic fluid are associated with the mother. If the pregnancy does not result in a live birth, fetal results should be attached to the mother's case.

- b. Pediatrician follow-up visits should be completed at delivery, 2, 6, 12, 18, and 24 months (if applicable, see time frame above). If a specific follow-up record is unavailable, a record collected within 2 months of the record needed may be used (i.e., 8-month record as a substitute for the 6-month record). Birth records should include the history and physical, discharge summary, lab reports, and any radiology reports, if available. Please check off what follow-up records have been collected in the Merlin extended data. Additional care will vary based on testing results of both the mother and infant and if abnormalities are detected in the infant at birth. Records for any specialty visits, ER visits diagnosing a new birth defect, or any follow-up diagnostic procedures should also be collected. Infant follow-up should still occur regardless of whether the infant has any identifiable abnormalities or negative test results. In the event of a fetal loss, attach the records to the mother's case.
- c. The Pregnancy Registry Coordinators or medical record abstractors at the State office may reach out for additional records/follow-up as needed.

D. Linking pregnant women and infants to care

1. Linking women to prenatal care
 - a. At the time of interview, ask the pregnant woman if she is receiving prenatal care. If a pregnant woman is not in care, make a referral to Healthy Start.
 - b. The CHD Maternal and Child Health Zika Contact will refer the woman to Healthy Start and/or a prenatal provider. The role of this contact is outlined in the document entitled Expectations and Resources for Local Department of Health Staff Serving as a Point-of-Contact for Pregnant Women and Infants with Zika, which is available in the [List of Appendices](#). Please contact the Arbovirus Surveillance Coordinator or Pregnancy Registry Coordinator if you are unsure who the internal Zika maternal and child health contact is for your county.
 - c. The Communication Tool for County Maternal and Child Health Zika Point-of-Contact and Epidemiology is located in the [List of Appendices](#).
2. Healthy Start
 - a. Pregnant women and infants until the age of three.
 - b. All pregnant women should receive a referral to the Healthy Start program (unless she declines or was previously referred). At the time of interview, ask the pregnant woman if she has previously been referred to Healthy Start. If she has not or if she is not sure, inform her of the Healthy Start services available and refer her to the appropriate contact to get additional information. Record this information in Merlin. For more information on the Healthy Start program, please visit www.floridahealth.gov/programs-and-services/childrens-health/healthy-start/#heading_1.
 - c. Review this document for the Healthy Start Coalition information for your county: Zika Healthy Start Coalition Directory, which is available at www.floridahealth.gov/diseases-and-conditions/mosquito-borne-diseases/_documents/zika-healthy-start-coalition-directory.docx.
3. Early Steps

- a. Birth through 36 months.
- b. Serves families with infants and toddlers who have developmental delays or an established condition likely to result in a developmental delay. Microcephaly was added to the list of conditions as well as infants with positive Zika test results after birth. Infants potentially meeting Early Steps criteria should also be referred after birth and the information filled out in the infant case in Merlin. Check to see if the mother received information and a referral to Early Steps. If she has not or is she is not sure, inform her of the Early Steps services available and refer her to the appropriate contact to get additional information. For more information on Early Steps, please visit www.floridahealth.gov/programs-and-services/childrens-health/healthy-start/#heading_1.
- c. Visit this link for the Early Steps contact for your county:
www.floridahealth.gov/diseases-and-conditions/mosquito-borne-diseases/_documents/early-steps-contact-list.pdf.

7. CONTROLLING FURTHER SPREAD

A. Patient and household education on prevention recommendations

1. Awareness of mosquito-borne diseases
2. Drain standing water to stop mosquitoes from multiplying.
 - a. Drain water from garbage cans, house gutters, buckets, pool covers, coolers, toys, flowerpots, or any other containers where sprinkler or rainwater has collected.
 - b. Discard old tires, drums, bottles, cans, pots and pans, broken appliances, and other items that are not being used.
 - c. Empty and clean birdbaths and pet water bowls at least once or twice a week.
 - d. Protect boats and vehicles from rain with tarps that do not accumulate water.
 - e. Maintain swimming pools in good condition and appropriately chlorinate. Empty plastic swimming pools when not in use.
3. Cover skin with clothing or repellent
 - a. CLOTHING: Wear shoes, socks, and long pants and long sleeves. This type of protection may be necessary for people who must work in areas where mosquitoes are present.
 - b. REPELLENT: Apply EPA-registered mosquito repellent to bare skin and clothing. Do not use insect repellent on infants younger than 2 months old.
 - c. Always use repellents according to the label. Repellents with DEET, picaridin, oil of lemon eucalyptus, *para*-menthane-diol, and IR3535 are effective. See the repellent frequently asked questions document in the [List of Appendices](#) for more information.
 - d. Use mosquito netting to protect children younger than 2 months old, being sure to position the netting in a way to prevent tangling of the infant.
4. Cover doors and windows with intact screens to keep mosquitoes out of the house and repair broken screening on windows, doors, porches, and patios.
5. Use condoms consistently and correctly or abstain from sex.
 - a. At least eight weeks after possible exposure for women and at least three months after possible exposure for men.
 - b. Pregnant women and their sexual partners should follow these guidelines for the duration of the pregnancy.

B. Environmental evaluation

In the event of a locally acquired Zika case or outbreak, local mosquito control or environmental health personnel may conduct an immediate assessment of the household. A Mosquito Control Environmental Assessment Form template can be found at the following link: www.floridahealth.gov/diseases-and-conditions/mosquito-borne-diseases/_documents/mosquito-control-environmental-assessment-form.docx. Determining the vector species involved in transmission is important (*Ae. aegypti* or *Ae. albopictus*). Additional information on the control of these two species can be found at www.floridahealth.gov/diseases-and-conditions/mosquito-borne-diseases/_documents/toolbox-for-control-of-aedes-aegypti-and-aedes-albopictus.pdf and www.cdc.gov/zika/public-health-partners/vector-control-us.html.

C. Issue a mosquito-borne illness advisory or alert as necessary

The need for mosquito-borne illness advisories and alerts is determined by the CHD Director or Administrator after consultation with local mosquito control experts and DOH Central Office using the below criteria. See [Chapter 11](#) of the guide for more detailed information. Press or media releases are **not** recommended for imported mosquito-borne disease infections.

1. **Advisory criteria: one locally acquired case**
2. **Alert criteria: a cluster of two or more locally acquired, confirmed cases and/or blood donors**
3. A public health emergency may be declared upon identification of imported/locally acquired Zika cases at the discretion of the State Surgeon General.

Templates for both advisories or alerts can be found in the [List of Appendices](#). Templates are available in both English and Spanish.

D. Education

1. Education messages should be targeted to at-risk populations (e.g., pregnant women, immigrant populations, outdoor workers, tribal representatives, homeless people) in languages appropriate to the local population. Media should be used, including radio, newspaper, and television public service announcements.
2. Educational materials and fact sheets should be provided in English and in appropriate languages if there are immigrant populations in affected area. Some educational materials are available at the following link: www.floridahealth.gov/diseases-and-conditions/mosquito-borne-diseases/educational-materials.html.
3. The Environmental Public Health Tracking Program has created census tract level maps designed to identify at-risk populations. Previous work on local dengue virus transmission in Key West identified several variables that put an individual at increased risk of not receiving prevention messaging, including populations that were non-white, did not speak English at home, and had low socioeconomic status. These risk maps combine these variables with women of childbearing age (relevant for ZIKV messaging) to develop a composite index value of risk. The maps can help to drive CHD outreach and education activities: <http://hermes.freac.fsu.edu/che/zika>.
4. Encourage residents to always assist in the effort to eliminate artificial container habitats to prevent breeding of *Aedes* mosquitoes, which transmit ZIKV, as appropriate when a local mosquito-borne disease infection is confirmed.

5. Post an EpiCom message indicating the details of locally acquired cases. Posts are not needed for imported cases unless there is an unusual case, a cluster of travelers, etc.
6. Distribute information to local health care providers about clinical signs and symptoms of Zika when CDC or DOH issues a Health Alert Network (HAN) or there are unusual numbers of imported cases or an increased trend of imported cases compared to baseline for the county. For locally acquired cases, see [section 7C](#) above for additional actions. A “Zika Fever – Information for Clinicians” document can be found in the [List of Appendices](#). Review the Florida Weekly Arbovirus Surveillance report for current arboviral activity in Florida. The report is located at www.floridahealth.gov/diseases-and-conditions/mosquito-borne-diseases/surveillance.html.

8. IMPORTANT LINKS

- A. Florida Confidential Zika Virus Case Report Form
www.floridahealth.gov/diseases-and-conditions/disease-reporting-and-management/disease-reporting-and-surveillance/_documents/crf-zika-confidential.pdf
- B. Florida Department of Health Zika Webpage
www.zikafreefl.org/
- C. Florida Zika Action Plan
www.floridahealth.gov/diseases-and-conditions/mosquito-borne-diseases/_documents/zika-playbook.pdf
- D. CDC Zika information
www.cdc.gov/zika/
- E. CDC Zika Reports
www.cdc.gov/mmwr/zika_reports.html
- F. Surveillance and Control of Selected Mosquito-Borne Diseases in Florida Guidebook
<http://www.floridahealth.gov/diseases-and-conditions/mosquito-borne-diseases/guidebook.html>
- G. Mosquito-borne Disease Surveillance Reports
www.floridahealth.gov/diseases-and-conditions/mosquito-borne-diseases/surveillance.html
- H. CDC FAQ: Insect Repellent Use and Safety
www.cdc.gov/westnile/faq/repellent.html
- I. Florida Resident’s Guide to Mosquito Control
www.floridahealth.gov/diseases-and-conditions/mosquito-borne-diseases/_documents/fl-resident-guide-to-mosquito-control-ifas.pdf

9. REFERENCES

- A. Heymann, D.L. (Ed.). (2015). *Control of Communicable Diseases Manual* (20th ed.). Washington: American Public Health Association.
- B. American Academy of Pediatrics. (2018). *Red Book: 2018 Report of the Committee on Infectious Diseases* (31st ed.). Grove Village, IL: American Academy of Pediatrics.
- C. Division of Disease Control and Health Protection. (2018). *Surveillance and Control of Selected Mosquito-Borne Diseases in Florida Guidebook*. Tallahassee, FL: Florida Department of Health.