



Zika Fever:

Sample Submission Guidance for County Health Departments (CHDs)

Version 2.0 (June 7, 2016)

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Notification if there is a suspected case of Zika fever:

- County health departments (CHDs) should immediately inform their local mosquito control district of suspect imported and locally acquired Zika fever or other mosquito-borne disease cases who were in the county during the viremic stage of illness (first week of illness).
- **CHDs should immediately contact Dr. Andrea Bingham (850-245-4444 ext. 3425) or Dr. Danielle Stanek (850-245-4117) if any persons under investigation (PUIs) are suspected to have Guillain-Barre Syndrome (GBS) or are suspected locally acquired cases, as well as any infants or pregnant women who test positive for Zika virus at state or commercial laboratories.**

Recommended testing for Zika virus:

- Testing using urine real time reverse-transcriptase polymerase chain reaction (RT-PCR) is now recommended rather than serum RT-PCR for acute Zika fever per Centers for Disease Control and Prevention (CDC).
- Serum collection is recommended for Zika IgM virus antibody testing and to test for other arboviruses with similar symptoms such as dengue or chikungunya.
- Zika virus IgM antibody testing is the recommended test for potentially exposed asymptomatic pregnant women.
- CDC Health Update. Diagnostic Testing of Urine Specimens for Suspected Zika Virus Infection, May 25, 2016. <http://emergency.cdc.gov/han/han00389.asp>
- Interim Guidance for Interpretation of Zika Virus Antibody Test Results. MMWR; June 3, 2016 / 65(21) http://www.cdc.gov/mmwr/volumes/65/wr/mm6521e1.htm?s_cid=mm6521e1_w

Commercial testing for Zika virus:

- Serum and urine Zika virus RT-PCR testing is now available through Quest Diagnostics and LabCorp. A Zika RT-PCR kit may be available in hospitals soon.
- Quest Diagnostics has a fee schedule for insured (\$500) and uninsured (\$120) patients. Zika virus IgM antibody testing is not yet commercially available.
- **Samples that test positive or equivocal at Quest Diagnostics or LabCorp should be forwarded to Bureau of Public Health Laboratories (BPHL)-Tampa or BPHL-Jacksonville for confirmatory Zika virus testing.**
- **Only Zika virus testing performed at BPHL, CDC, or other reference laboratories is recognized for public health surveillance purposes.**

Zika virus testing at BPHL is recommended for patients meeting any of the following criteria:

- Samples from patients with positive or equivocal commercial laboratory results
- Testing not available at commercial laboratories including Zika IgM serology
- Zika virus screening tests (including RT-PCR) are also available at BPHL for patients who are uninsured or otherwise do not have financial resources for Zika virus testing at commercial laboratories and who meet any of the following criteria:

- 1) Requested by a health care provider
AND
Pregnant women who, while pregnant, **traveled to an area reporting Zika virus activity** regardless of the length of time since the travel/illness occurred, but ideally within 2-12 weeks of travel
- 2) Requested by a health care provider
AND
All persons, including pregnant women, with two or more of the following signs/symptoms: fever, maculopapular rash, arthralgia, or conjunctivitis **and** a history of travel to an area reporting Zika virus activity in the two weeks prior to illness onset
- 3) Requested by a health care provider
AND
Mothers of an infant or fetus with microcephaly and/or intracranial calcifications, or poor fetal outcome diagnosed after the first trimester **and** with history of travel to an area with Zika virus activity during pregnancy **or** sexual contact with a potentially infected male during pregnancy
- 4) Requested by a health care provider
AND
Any person with three or more of the following signs/symptoms: fever, maculopapular rash, arthralgia, or conjunctivitis with no travel **and** no epidemiologic linkages to persons potentially infected with Zika virus (suspected local case)

Criteria for testing of suspect local cases at BPHL (i.e. no history of travel):

- Persons with compatible symptoms and who report household members or other close contacts have traveled to an area reporting Zika virus activity in the month prior to onset
- Non-pregnant persons with two compatible symptoms and who report sexual contact in the past four weeks with a person who had recent travel to a Zika active area
- Pregnant women who report sexual contact in the past four weeks with a person who had recent travel to a Zika active area and whom one of the two partners reports at least one of the four major symptoms during travel or in the two weeks afterwards.
- Persons with no epidemiologic linkages to potentially exposed sexual contacts or other travelers, but who have at least three of the following symptoms (rash, fever, arthralgia, conjunctivitis)
- Ensure other testing has been done to rule out more common illnesses such as influenza, *Streptococcus*, etc.
- Obtain information regarding recent outside activities or if other contacts have similar symptoms.

PCR testing:

- Samples recommended to be collected from symptomatic people within the first 21 days of illness:
 - Serum (**2 ml serum/red or tiger top tube**)
AND
 - Urine sample collected (**2-3 ml collected in a sterile container**).
- Other samples that may be tested using PCR if available: CSF, amniotic fluid, birth cord blood, and tissues (placenta, umbilical cord, fetal tissues, etc.). Please contact Dr. Bingham or Dr. Stanek immediately for any requests to submit these samples.

- Asymptomatic pregnant women with travel to Zika virus active areas should be tested using Zika IgM serology.
- **Depending on quantity of samples going to BPHL**, samples meeting the requirements for Zika fever PCR testing at BPHL will also be considered for tested for dengue and chikungunya **if the patient reported fever**.
- **Depending on quantity of samples going to BPHL**, samples collected during the acute stage of illness and meeting standard requirements for dengue and chikungunya testing will also be tested for Zika virus if travel to an area with Zika virus activity is reported.

IgM antibody and serum neutralization testing:

- Zika virus PCR is the definitive test. If PCR testing is positive, Zika virus IgM testing is not necessary.
- If PCR is negative on samples collected <5 days post-symptom onset, no further testing is needed for non-pregnant persons. Zika virus IgM testing should be conducted on a sample collected at least 8 days after illness onset in cases involving pregnant women with negative PCR tests in the first 5 days following illness onset.
- Acute serum samples should be collected ≤ 8 days after illness onset; convalescent serum samples should be collected > 8 days after illness onset. **(2 ml serum/red or tiger top tube for each sample)**.
- Plaque reduction neutralization testing (PRNT) will be required for cases who have negative Zika virus PCR results, but test positive for Zika virus IgM antibody. This testing is needed to confirm that the IgM is not a false positive result. CDC currently performs all Zika virus PRNT testing. However BPHL is expected to have this testing capacity in the next few months.
- 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.

Ordering and shipping:

- **Please initiate a Merlin case at the time BPHL or CDC testing is requested (if testing criteria are met). Critical information to include in Merlin ASAP: travel history including dates and location, symptoms, pregnancy status, hospitalization status, and type of reporter who notified CHD of the PUI.**
- PCR testing for Zika virus at BPHL can be ordered using test code 1680 (Arbovirus PCR) in the virology section of the standard BPHL form and write "Zika PCR" in the comment box. IgM serologic tests can be ordered using test code 1500 Arbovirus IgM Antibody. **Be sure to include onset, sample collection date, pregnancy status (female patients), travel country/territory and dates, whether fever was reported, and Merlin number on the lab form.**
- List the CHD epidemiologist as the submitter in order to receive laboratory results rapidly. You will need to ensure the provider also gets a copy.
- Please notify BPHL before submitting the sample for high priority PUI (fetal microcephaly, intracranial calcifications, infant birth defects, poor pregnancy outcomes after the first trimester of pregnancy, suspect GBS, or suspect local cases).
- Ship 2 ml of serum (red or tiger top blood collection tube) kept chilled and shipped overnight to BPHL as for dengue and chikungunya samples. Ship 2-3 ml of urine using the same method.
- Serum should be separated from red blood cell component before shipping to prevent hemolysis.
- Providers who partner with local hospitals may wish to check availability of blood drawing and courier service to assist with sample collection and delivery.

Pregnant Women and sexual transmission guidance:

- Pregnant women and their male partners should be made aware that abstinence or consistent and correct use of condoms during sex (i.e. vaginal intercourse, anal intercourse, or fellatio) is recommended for the duration of the pregnancy if the male partner was potentially exposed to Zika virus during the pregnancy. Testing of male partners potentially exposed to Zika virus to assess risk is not routinely recommended.

Share with clinicians evaluating pregnant women or women planning to become pregnant:

- Interim Guidelines for Pregnant Women during a Zika Virus Outbreak — United States, 2016. MMWR; January 19, 2016 / 65(2);1–4: <http://www.cdc.gov/mmwr/volumes/65/wr/mm6502e1er.htm>
- Update: Interim Guidelines for Health Care Providers Caring for Pregnant Women and Women of Reproductive Age with Possible Zika Virus Exposure—United States, 2016. MMWR; February 5, 2016 / 65:1-6.
- Update: Interim Guidance for Health Care Providers Caring for Women of Reproductive Age with Possible Zika Virus Exposure — United States, 2016. MMWR; April 1, 2016 / 65(12);315-322. http://www.cdc.gov/mmwr/volumes/65/wr/mm6512e2.htm?s_cid=mm6512e2_w
- Interim Guidelines for Prevention of Sexual Transmission of Zika Virus—United States, 2016. MMWR; February 5, 2016 / 65:1-2.
- Update: Interim Guidance for Prevention of Sexual Transmission of Zika Virus — United States, 2016. MMWR; April 1, 2016 / 65(12);323-325. http://www.cdc.gov/mmwr/volumes/65/wr/mm6512e3.htm?s_cid=mm6512e3_w

Share with clinicians evaluating potentially infected infants:

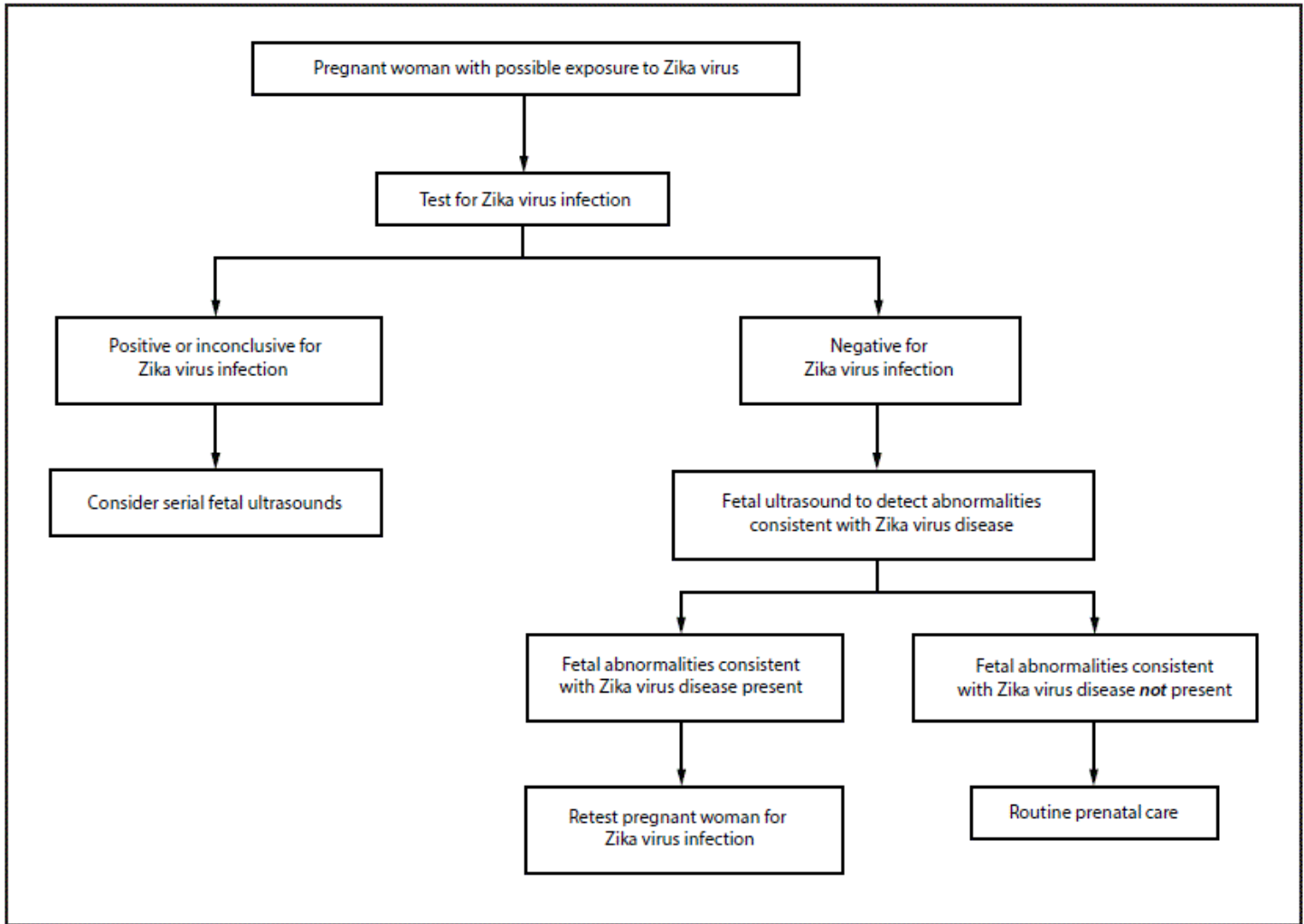
- Interim Guidelines for the Evaluation and Testing of Infants with Possible Congenital Zika Virus Infection — United States, 2016. MMWR; January 29, 2016 / 65(3):65-67. <http://www.cdc.gov/mmwr/volumes/65/wr/mm6503e3.htm>
- Update: Interim Guidelines for Health Care Providers Caring for Infants and Children with Possible Zika Virus Infection — United States, February 2016. MMWR; February 26, 2016 / 65(7):182-187. http://www.cdc.gov/mmwr/volumes/65/wr/mm6507e1.htm?s_cid=mm6507e1_w

For complete and up-to-date listing of all CDC Zika-related MMWR articles please see:

http://www.cdc.gov/mmwr/zika_reports.html

Useful algorithms for clinicians evaluating pregnant women who traveled to areas with Zika virus activity (Figure 1) or who reside in areas with ongoing Zika virus activity (Figure 2) taken from the April 1, 2016 CDC MMWR: http://www.cdc.gov/mmwr/volumes/65/wr/mm6512e2.htm?s_cid=mm6512e2_w.htm

FIGURE 1. Updated interim guidance: testing algorithm^{*,†,§,¶} for a pregnant woman with possible Zika virus exposure,^{} not residing in an area with active Zika virus transmission**



* Testing is recommended for pregnant women with clinical illness consistent with Zika virus disease, including one or more of the following signs or symptoms: acute onset of fever, rash, arthralgia, or conjunctivitis during or within 2 weeks of travel or possible sexual exposure. Testing includes Zika virus reverse transcription-polymerase chain reaction (RT-PCR), and Zika virus immunoglobulin M (IgM) and neutralizing antibodies on serum specimens. More information is available at <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6512e2.htm>. Because of the overlap of symptoms and areas where other viral illnesses are endemic, evaluate for possible dengue or chikungunya virus infection.

† Testing can be offered to pregnant women without clinical illness consistent with Zika virus disease. If performed, testing should include Zika virus IgM, and if IgM test result is positive or indeterminate, neutralizing antibodies on serum specimens. Testing should be performed 2–12 weeks after travel.

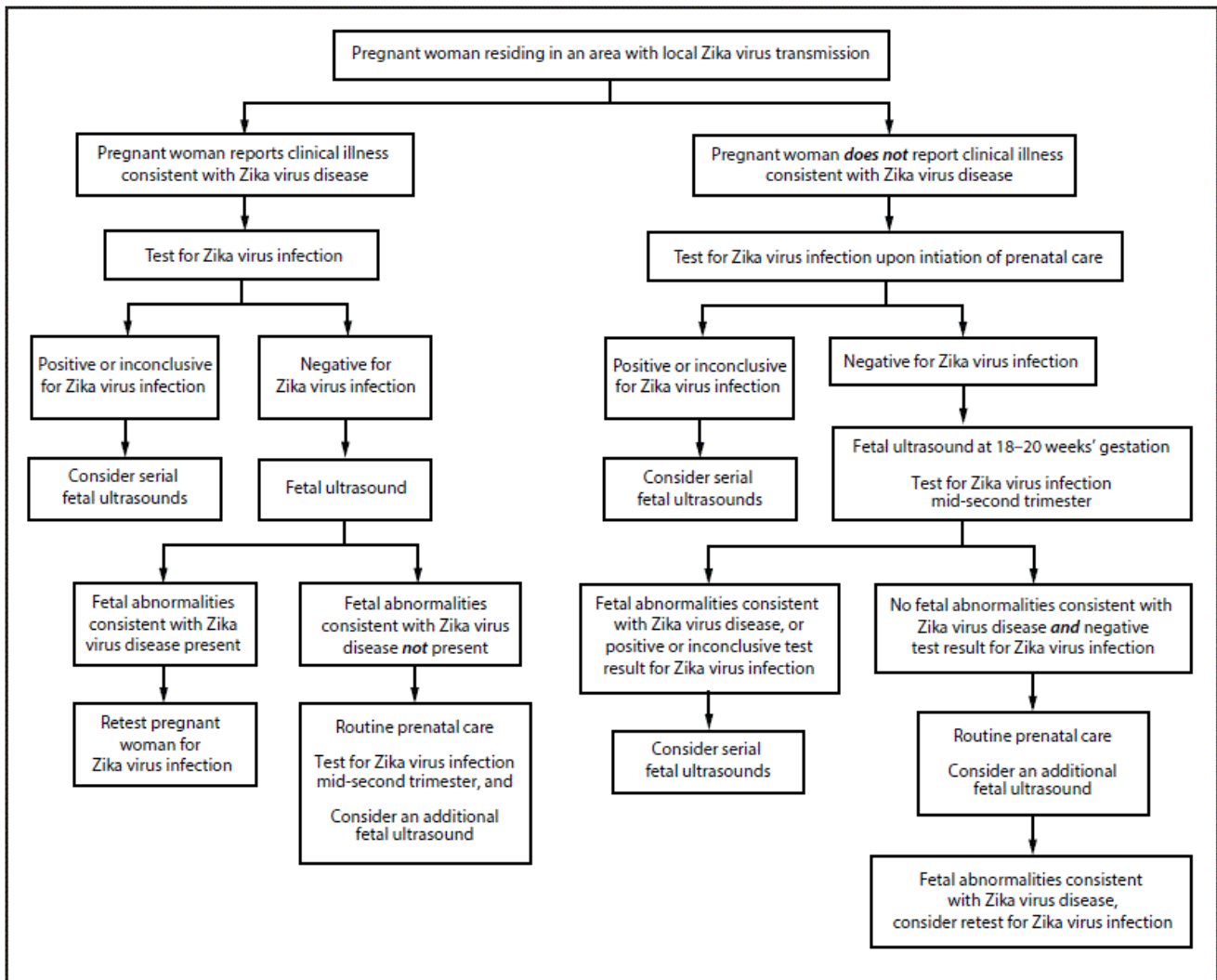
§ Laboratory evidence of maternal Zika virus infection: 1) Zika virus RNA detected by RT-PCR in any clinical specimen; or 2) positive Zika virus IgM with confirmatory neutralizing antibody titers that are ≥4-fold higher than

dengue virus neutralizing antibody titers in serum. Testing is considered inconclusive if Zika virus neutralizing antibody titers are <4-fold higher than dengue virus neutralizing antibody titers.

† Fetal abnormalities consistent with Zika virus disease include microcephaly, intracranial calcifications, and brain and eye abnormalities. Fetal ultrasounds might not detect abnormalities until late second or early third trimester of pregnancy.

** Possible exposure to Zika virus includes travel to an area with active Zika virus transmission (<http://wwwnc.cdc.gov/travel/notices/>), or sex (vaginal intercourse, anal intercourse, or fellatio) without a condom with a man who traveled to, or resided in, an area with active Zika virus transmission. Testing is not currently recommended for pregnant women with possible sexual exposure to Zika virus if both partners are asymptomatic.

FIGURE 2. Updated interim guidance: testing algorithm*†,§,¶ for a pregnant women residing in an area with active Zika virus transmission, with or without clinical illness †† consistent with Zika virus disease**



* Tests for pregnant women with clinical illness consistent with Zika virus disease include Zika virus reverse transcription-polymerase chain reaction (RT-PCR), and Zika virus immunoglobulin M (IgM) and neutralizing antibodies on serum specimens. More information is available at http://www.aphl.org/Materials/CDCMemo_Zika_Chik_Deng_Testing_011916.pdf. Because of the overlap of symptoms and areas where other viral illnesses are endemic, evaluate for possible dengue or chikungunya virus infection. If chikungunya or dengue virus RNA is detected, treat in accordance with existing guidelines.

Timely recognition and supportive treatment for dengue virus infections can substantially lower the risk of medical complications and death. Repeat Zika virus testing during pregnancy is warranted if clinical illness consistent with Zika virus disease develops later in pregnancy.

† Testing can be offered to pregnant women without clinical illness consistent with Zika virus disease. If performed, testing should include Zika virus IgM, and if IgM test result is positive or indeterminate, neutralizing antibodies on serum specimens. Results from serologic testing are challenging to interpret in areas where residents have had previous exposure to other flaviviruses (e.g., dengue, yellow fever) because of cross-reactivity with other flaviviruses.

§ Laboratory evidence of maternal Zika virus infection: 1) Zika virus RNA detected by RT-PCR in any clinical specimen; or 2) positive Zika virus IgM with confirmatory neutralizing antibody titers that are ≥ 4 -fold higher than dengue virus neutralizing antibody titers in serum. Testing would be considered inconclusive if Zika virus neutralizing antibody titers are < 4 -fold higher than dengue virus neutralizing antibody titer.

¶ Fetal abnormalities consistent with Zika virus disease include microcephaly, intracranial calcifications, and brain and eye abnormalities. Fetal ultrasounds might not detect abnormalities until late second or early third trimester of pregnancy.

** <http://wwwnc.cdc.gov/travel/notices/>. Local health officials should determine when to implement testing of asymptomatic pregnant women based on information about levels of Zika virus transmission and laboratory capacity.

†† Clinical illness is consistent with Zika virus disease if one or more signs or symptoms (acute onset of fever, rash, arthralgia, or conjunctivitis) are present.