Allegheny County Health Department (ACHD) Zika Virus Protocol

1. Determine if testing is appropriate based on patient symptoms and exposure history. Currently, the following persons are eligible for testing:

   a. Pregnant women who have traveled to a Zika-affected area while pregnant or shortly before becoming pregnant (within 8 weeks of pregnancy), regardless of symptoms.

   b. Pregnant women who have had unprotected sex with a partner who has traveled to an area with active Zika virus transmission.

   c. Travelers to Zika-affected areas with at least one symptom of Zika virus infection (fever, maculopapular rash, arthralgia, nonpurulent conjunctivitis) within 2 weeks of travel

   d. Symptomatic sex partners of travelers with Zika symptoms or a positive Zika test

   e. Infants with microcephaly or intracranial calcifications born to women who traveled to or resided in an area with Zika virus transmission while pregnant, or infants born to mothers with positive or inconclusive test results for Zika virus infection

2. Contact ACHD to review testing criteria and receive approval for testing

   - Jen Fiddner 412-578-8002 jennifer.fiddner@alleghenycounty.us
   - Dr. Kristen Mertz 412-578-8323 kristen.mertz@alleghenycounty.us
   - Dr. LuAnn Brink 412-578-8354 luann.brink@alleghenycounty.us
   - Main ACHD Epi line 412-578-8066
   - ACHD main number (best for after hours) 412-687-2243

3. Once Zika testing is approved by ACHD, specimens should be sent to PA BOL. PA BOL will coordinate testing with the CDC laboratory. Shipment should be arranged so specimens arrive at PA BOL Monday through Friday. Overnight shipping is preferred.

   a. Collect 3 mL of serum for serology testing. Use a red-top or serum separator tube for specimen collection. Whole blood will not be accepted for serology testing. After centrifuging the specimen, aseptically transfer serum to a sterile tube.

   b. For serology testing, the specimen should be refrigerated at 4°C. The sample may be placed in an insulated container with ice packs. Additional ice packs should be used in the summer to ensure specimen integrity in hot weather

   c. If the patient is symptomatic, 0.5-1.0 mL of urine should be collected in addition to serum. Urine should be collected within 14 days of symptom onset. Serum should ideally be collected within 7 days of symptom onset (and can be collected within 14 days for pregnant women). The urine should be stored in a sterile screw top vial secured with thermoplastic self-sealing lab film. Ensure the seal is tight so there are no leaks. For rRT-PCR testing specimens should be kept cold (2–6 °C) or frozen (-70 °C) for storage and shipping.

ACHD Zika Protocol June 30, 2016
d. Asymptomatic pregnant women evaluated < 2 weeks after possible Zika virus exposure should be offered serum and urine testing via rRT-PCR. If negative, these women should return 2-12 weeks after possible Zika virus exposure and offered Zika virus IgM antibody testing.

e. INFANTS: Zika virus RT-PCR and serology assays can be performed on infant serum or serum or plasma collected from the umbilical cord. If cerebrospinal fluid (CSF) specimens are available, Zika virus RT-PCR should be performed; however, CSF specimens should not be collected for the sole purpose of Zika virus testing. Other specimens that can be tested include the placenta and the umbilical cord. Histopathologic examination and immunohistochemical staining can be performed. Zika virus RT-PCR on fixed and frozen tissue should also be considered. Please contact ACHD for instructions. More detailed information is available on CDC website: http://www.cdc.gov/zika/hc-providers/tissue-collection-submission.html

4. A PA BOL Zika Virus Laboratory Submission Form must be completed and accompany Zika specimens. The CDC will not process specimens with incomplete forms. ACHD will e-mail or fax the appropriate form to the provider once testing is approved.

a. Onset date, symptoms and travel history are required for testing. These sections must be completed on the form.

b. Pregnancy status must be documented in the brief Clinical Summary field of the form.

c. Document if patient has ever been vaccinated for or has a history of yellow fever, Japanese encephalitis or tickborne encephalitis viruses under the “Relevant Immunization History” section.

d. The submitting lab should be listed in the “original submitter” section. Additional submitters can be added on the back of the form.

5. Zika specimens should be shipped to:

   PA Bureau of Laboratories
   Attention: Lisa Dettinger/Dr. Annette Regec
   110 Pickering Way
   Exton, PA 19341-1310

a. Overnight delivery is encouraged with proper packaging of specimens

b. Specimens should arrive at PA BOL Monday through Friday

6. Notify ACHD when the specimen ships to PA BOL. ACHD will alert PA BOL.

7. ACHD and the submitter will be notified of test results in approximately 3-4 weeks. CDC will also test for Dengue if the patient is symptomatic.

8. Zika virus testing at commercial labs is now available for symptomatic persons using PCR assays. Providers should be aware that commercial laboratories performing PCR testing do not also offer Zika IgM ELISA or confirmatory serologic testing (plaque reduction neutralization test, or PRNT). Therefore, providers should store a serum aliquot for subsequent Zika IgM ELISA testing if the rRT-PCR assay is negative. Please contact ACHD to send specimen to PA BOL for antibody testing.

9. Dengue and chikungunya testing at a commercial laboratory should also be considered for symptomatic patients if results are needed sooner than 3-4 weeks. The following laboratories test for dengue and chikungunya. NSAIDS should be avoided until dengue is ruled out (risk for hemorrhage).
a. Chikungunya:
   i. Focus Diagnostics (RT-PCR, IgM, IgG). Refrigerated serum is acceptable for RT-PCR. Ship on cold packs.
   ii. ARUP Laboratories (IgM, IgG). Refrigerated serum is acceptable for IgM, IgG. Ship on cold packs.
   iii. PA BOL (PCR). Refrigerated serum collected within 7 days of symptom onset. Ship on cold packs.

b. Dengue:
   i. Focus Diagnostics (IgM)
   ii. ARUP Laboratories (IgM, IgG)
   iii. Quest Diagnostics (IgM, IgG). Serum samples are preferred for most dengue tests. The blood sample should be taken in a red-top or tiger-top tube. Citrate (collected in yellow top tubes) and heparin plasma (green top tubes) can be tested by RT-PCR. After blood is allowed to clot, separate serum by centrifugation and keep serum refrigerated at 4°C or frozen at -20°C.
   iv. PA BOL (PCR). Refrigerated serum collected within 7 days of symptom onset. Ship on cold packs.

10. Counsel symptomatic patients to avoid unprotected sex, mosquito exposure and blood/sperm/tissue donation until test results are received. Counsel travelers to avoid mosquitos in Allegheny County for 3 weeks after their return to prevent infection of local mosquitos, even if travelers are asymptomatic.

   a. Couples trying to get pregnant should wait 2 months after either partner has traveled or 6 months if a male has traveled and has had symptoms. Testing asymptomatic travelers before trying to conceive is not indicated at this time.
   b. Men who have traveled to a Zika-affected area and have a pregnant partner should either abstain from sex (vaginal, anal, or oral (mouth-to-penis)) or use a condom for the duration of the pregnancy.

Relevant guidance:

a) Interim Guidance for Healthcare Providers Caring for Pregnant Women with Possible Zika Virus Exposure (7/29/2016)
   http://www.cdc.gov/mmwr/volumes/65/wr/mm6529e1.htm?s_cid=mm6529e1_e

b) Interim Guidance for Healthcare providers caring for Women of Reproductive Age with Possible Zika Virus Exposure (3/25/16)
   http://www.cdc.gov/mmwr/volumes/65/wr/mm6512e2er.htm?s_cid=mm6512e2er_w

c) Interim Guidelines for the Evaluation and Testing of Infants with Possible Congenital Zika Virus Infection (2/19/2016)
   http://www.cdc.gov/mmwr/volumes/65/wr/mm6507e1er.htm?s_cid=mm6507e1.htm_w

d) Interim Guidelines for Prevention of Sexual Transmission of Zika Virus (7/25/16)
   http://www.cdc.gov/mmwr/volumes/65/wr/mm6529e2.htm?s_cid=mm6529e2_e

e) Updated diagnostic testing for Zika, chikungunya, and dengue viruses in US Public Health Laboratories

ACHD Zika Protocol June 30, 2016