Florida Department of Health

Zika Virus **Laboratory Diagnosis**	**Focus Area: Collection, Packaging and Shipping of Laboratory Specimens**

Guidance document number 2016-02

Zika Virus Diagnostic Specimen Collection, Packaging and Shipping Guidance for Laboratories and County Health Departments

Version 4.1 December 2018

Summary:

- **Authorization:** Contact your local County Health Department for authorization prior to collecting and shipping diagnostic specimens for Zika virus testing. **Testing requires prior approval before specimen submission.**

- **Specimen Selection:** Specimens for rRT-PCR are preferred. Serum for IgM testing can also be collected. Other specimens may also be appropriate in consultation with epidemiology.

- **Specimen Collection:** Specimens for rRT-PCR testing should be collected within the first 14 days of illness: serum (**2 ml serum/red or red/grey marble tiger top tube)** AND urine sample collected **(1–2 ml, 5 ml max, in a sterile tube).** Serum and urine samples are preferred.

- **Packaging: Transport by Commercial Carrier:** t is recommended that all suspect Zika virus diagnostic specimens be packaged according to IATA/DOT Packaging Instructions 650 for Biological substance, Category B agents which requires specimens be packed in a basic triple packaging system with a primary watertight container wrapped with absorbent material, secondary watertight container and an outer shipping package.

- **Packaging: Transport by Local Courier:** As above, specimens should be packaged in a basic triple packaging system which consists of a primary watertight container wrapped with absorbent material, secondary watertight container and an outer shipping package.

- **Laboratory Requisition:** 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. For county health departments using HMS, order test code 1539 (Arbo ZIKA IgM ELISA) and test code 1550 (Arbo TRIOPLEX). Be sure to include symptom onset, sample collection date, pregnancy status (female patients), travel country/territory and dates, and whether fever was reported on all orders.

- **Shipping to Jacksonville BPHL:**

  Bureau of Public Health Laboratories-Jacksonville
  1217 N. Pearl Street
  Jacksonville, FL 32202
  Attention: Valerie Mock

  For Additional Information: Contact Valerie Mock at 904-791-1539 or 904-637-9284
  Alternate contact Pamela Colarusso at 904-791-1540

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- **Shipping to Tampa BPHL:**

  Bureau of Public Health Laboratories-Tampa
  3602 Spectrum Blvd
  Tampa, FL 33612
  Attention: Lea Heberlein-Larson

  For Additional Information: Contact Lea Heberlein-Larson at 813-233-2307
  or 813-967-0330.
  Alternate contact Alexis LaCrue at 813-233-2315

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- **Shipping to Miami BPHL:**

Bureau of Public Health Laboratories-Miami
1325 NW 14th Ave
Miami, FL 33125
Attention: Stephen White

For Additional Information: Contact Stephen White at 305-325-2538
or 305-409-9925.
Alternate contact Darryl Pronty at 305-325-2537 or 305-797-5882

- Specimens delivered to Miami BPHL that require testing not available at Miami will be shipped overnight to Tampa BPHL or Jacksonville BPHL.

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Authorization

- Contact your local County Health Department prior to collecting any specimens from suspect Zika cases. **Testing requires prior approval before specimen submission**.

Specimen Selection

- Serum and urine specimens for rRT-PCR and IgM testing are preferred if collected <14 days following symptom onset.
- Serum only for IgM testing is preferred if collected ≥14 days following symptom onset.
- FOR PREGNANT WOMEN ONLY, serum and urine specimens for rRT-PCR and IgM testing are preferred regardless of time of collection following symptom onset or exposure.
- Other specimen types such as whole blood (EDTA), CSF, and amniotic fluid may be tested with the appropriate approvals from the local county health department.
- Urine, whole blood (EDTA), CSF, and amniotic fluid specimens MUST be submitted with a patient-matched serum specimen.
- If the appropriate specimen types or a patient-matched serum specimen are unobtainable for a high priority case (pregnant women with fetal abnormalities, infant birth defects or poor pregnancy outcomes after the first trimester of pregnancy with history of travel during pregnancy; suspect Guillain-Barre Syndrome patients with history of travel in the two weeks prior to illness onset; or suspect local cases), contact your local county health department to seek guidance.

PCR testing of serum and urine; or whole blood (EDTA), CSF, and amniotic fluid at Bureau of Public Health Laboratories (BPHL)-Tampa, BPHL-Jacksonville, or BPHL-Miami

- Samples collected <14 days following symptom onset or exposure: serum **(2 ml serum/red or tiger top tube)** AND urine sample collected **(1–2 ml, 5 ml max, in a sterile tube).** Serum and urine samples are preferred.
- Samples collected ≥14 days following symptom onset or exposure: serum (**2 ml serum/red or tiger top tube)** AND urine sample collected **(1–2 ml, 5 ml max, in a sterile tube). PCR testing will only be performed if serological tests are positive and the patient is pregnant or has no travel.** Samples (serum, whole blood, and CSF only) meeting the requirements for Zika PCR testing at BPHL will also be tested for dengue and chikungunya.
- **Depending on BPHL sample volume**, samples collected during the acute stage of illness and meeting standard requirements for dengue and chikungunya testing will also be tested for Zika virus by PCR if travel was to an area with reported Zika virus activity.
- Other specimen types such as whole blood (EDTA), CSF collected <14 days following symptom onset or exposure and amniotic fluid may be tested with the appropriate approvals from your local county health department. Note: pathology specimens, such as formalin fixed placental and fetal tissues from a birth, may also be collected and sent to BPHL. These samples will be referred to the CDC's Infectious Disease Pathology Branch for testing as appropriate.
- If the appropriate specimen types cannot be obtained from a high priority case (pregnant women with fetal abnormalities, infant birth defects or poor pregnancy outcomes after the first trimester of pregnancy with history of travel during pregnancy; suspect Guillain-Barre Syndrome patients with history of travel in the two weeks prior to illness onset; or suspect local

cases), contact your local county health department to seek guidance.
- Commercial testing for Zika virus PCR is currently available.

IgM antibody testing at Bureau of Public Health Laboratories (BPHL)-Tampa or BPHL-Jacksonville and Plaque Reduction Neutralization Test (PRNT, also referred to as serum neutralization test) at CDC:

- PCR is the definitive test. If PCR is positive on **serum, urine, whole blood, or CSF** serologic testing is not necessary, unless the patient is pregnant or has no history of travel.
- Acute serum samples should be collected <14 days following symptom onset**;** convalescent serum samples should be collected ≥14 days following symptom onset. (**2 ml serum/red or red/grey marble tiger top tube for each sample)**
- 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.
- Additional serological testing such as PRNT/serum neutralization can be performed at CDC to provide more definitive results.
- Commercial testing for Zika virus IgM is currently available.

Specimen Collection

- Specimen collection should be performed following standard precautions. Wear appropriate personal protective equipment (PPE) at all times while collecting and handling specimens.
- Specimens for rRT-PCR: Serum (2 ml), urine (1–2 ml, 5 ml max), whole blood (EDTA, 1–2 ml, 5 ml max), CSF (0.5–2 ml), and amniotic fluid (1–2 ml) may be used. Use serum separator tubes (red/grey marble tiger top tubes) centrifuged prior to shipment or tubes without additives- a plain, red-top tube poured off after centrifugation into a sterile tube for serum. Use sterile tubes for urine, CSF, and amniotic fluid and seal or cap container(s) securely to avoid leakage and loss of specimen. **Please do not submit urine in urine collection cups as these tend to leak during transport. Urine should be transferred to a sterile tube with a secure closure to prevent leakage in transport. An example of a preferred urine tube is the BD vacutainer urine collection tube, BD #364979, Fisher Scientific catalog #14-375-138.**
- Specimens for IgM: Serum or CSF collected as described above for rRT-PCR.
- Please notify BPHL (see end of document for contact information) before submitting a high priority sample (pregnant women with fetal abnormalities, infant birth defects or poor pregnancy outcomes after the first trimester of pregnancy with history of travel during pregnancy; suspect Guillain-Barre Syndrome patients with history of travel in the two weeks prior to illness onset; or suspect local cases).
- Ship immediately on cold packs. If samples cannot be shipped immediately, store samples at 4°C until shipment. DO NOT FREEZE
- Infant specimen volumes: Please submit the requested serum volume indicated above, but if this is not possible a minimum of 0.5 ml may be submitted. Urine does not need to be submitted.
- Pathology specimens, such as formalin fixed placental and fetal tissues from a birth, may also be collected in a sterile container and sent to BPHL. These specimens will be referred to the CDC's Infectious Disease Pathology Branch for testing as appropriate. The volume of 10% buffered formalin used to fix tissues should be 10x the volume of tissue. The entire tissue is not needed. Seal or cap container(s) securely to avoid leakage and loss of specimen. Store fixed tissues only at room temperature. 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.

Packaging requirement: Transport by Commercial Carrier

Package specimens in combination packaging according to IATA/DOT regulations.

NOTE: Per these regulations anyone who handles, offers for transport, or transports specimens must be trained to do so.

General Requirements

- It is recommended that specimens are packaged as a Category B agent using IATA/DOT Packaging Instructions 650 for Category B Agents which requires specimens be packed in a basic triple packaging system with 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.
- Package on cold packs, with the exception of fixed placenta. Fixed placenta is shipped at room temperature.

Inner Packaging Requirements

- Primary receptacle(s) must be watertight. An example watertight primary receptacle is a tube sealed with parafilm (flexible plastic covering).
- Secondary receptacle must be watertight. An example watertight secondary receptacle is a zip closure bag. If using a zip closure bag, place only one specimen per bag.
- The primary or secondary receptacle must also be pressure compliant (95kPa). An example of a preferred pressure compliant secondary receptacle is Therapak 95kPa compliant transport bags, Fisher Scientific catalog #22-130-021.
- A common inner packaging is one non-watertight tube placed in a zip closure bag (the primary watertight receptacle). Multiple zip closure bags are then placed in one pressure compliant bag (the secondary watertight receptacle).
- An itemized list of the contents enclosed between the secondary receptacle and the outer packaging (overpack).
- Absorbent material must be placed between the primary receptacle(s) and secondary receptacle and be of sufficient quantity to absorb the entire contents of the primary receptacle(s).

Outer Packaging Requirements

- Package must be rigid.
- Package must be properly labeled with the UN 3373 marking, the shipping name, “Biological Substance, Category B”, and orientation labels.
- Package must pass required testing.

Packaging Requirements: Transport by Local Courier

Package specimens in combination packaging according to 49 CFR 173.6(a)(4).
www.gpo.gov/fdsys/pkg/CFR-2011-title49-vol2/pdf/CFR-2011-title49-vol2-sec173-6.pdf

NOTE: Per these regulations anyone who handles, offers for transport, or transports specimens must be trained to do so.

General Packaging Requirements

- Packaging must coincide with 29 CFR 1910.1030 and specimens should be packed in a basic triple packaging system with 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.
- Package on cold packs, with the exception of fixed placenta. Fixed placenta is shipped at room temperature.

Inner Packaging Requirements

- Primary receptacle(s) must be watertight. An example of a watertight primary receptacle is a tube sealed with parafilm (flexible plastic covering).
- Secondary receptacle must be watertight. An example watertight secondary receptacle is a zip closure bag. If using a zip closure bag, place only one specimen per bag. A common inner packaging is one non-watertight tube placed in a zip closure bag (the primary watertight receptacle). Multiple zip closure bags are then placed in one large zip closure bag (the secondary watertight receptacle).
- An itemized list of the contents enclosed between the secondary receptacle and the outer packaging.
- Absorbent material must be placed between the primary receptacle(s) and secondary receptacle and be of sufficient quantity to absorb the entire contents of the primary receptacle(s).

Outer Packaging Requirements

- Package must be rigid, with strong packaging, to secure against shifting.

Laboratory Requisition

- 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 (DH1847, 13/05) and write “Zika PCR” in the comment box.
- IgM serologic tests can be ordered using test code 1500 Arbovirus IgM Antibody in the
- virology section of the standard BPHL form (DH1847, 13/05) and write “Zika IgM in the
- comment box.
- For county health departments using HMS, order test code 1539 (Arbo ZIKA IgM ELISA) and test code 1550 (Arbo TRIOPLEX).
- **Be sure to include onset, sample collection date, pregnancy status (female patients), travel country/territory and dates, and whether fever was reported.**

Ship Specimens To: the appropriate BPHL location, addresses are located on page 2. Deliver or ship samples immediately, at least by next day mid-morning delivery. For high priority specimens (pregnant women with fetal abnormalities, infant birth defects or poor pregnancy outcomes after the first trimester of pregnancy with history of travel during pregnancy; suspect Guillain-Barre Syndrome patients with history of travel in the two weeks prior to illness onset; or suspect local), first overnight or early morning delivery can be selected. Note: there can be significant additional shipping costs associated with first overnight or early morning delivery.

Notification: On shipment of a high priority specimen (pregnant women with fetal abnormalities, infant birth defects or poor pregnancy outcomes after the first trimester of pregnancy with history of travel during pregnancy; suspect Guillain-Barre Syndrome patients with history of travel in the two weeks prior to illness onset; or suspect local) for a suspect Zika patient to BPHL, please notify Valerie Mock with the Jacksonville BPHL (see below for contact information)
or Lea Heberlein-Larson with the Tampa BPHL (see below for contact information) or Stephen White with the BPHL-Miami (see below for contact information).
Provide the shipping tracking number if being shipped commercially.

For Additional Information:
On Submitting Specimens for Zika Testing: Contact your local County Health Department.
Contacts for Laboratory Questions:

Jacksonville
Valerie Mock, Bureau of Public Health Laboratories at 904-791-1539 or 904-637-9284; Pamela Colarusso at 904-791-1540

Tampa
Lea Heberlein-Larson, Bureau of Public Health Laboratories at 813-233-2307 or 813-967-0330; Alexis LaCrue at 813-233-2315

Miami
Stephen White, Bureau of Public Health Laboratories at 305-325-2538 or 305-409-9925; Darryl Pronty at 305-325-2537 or 305-797-5882

For more information on DOT shipping regulations:
https://hazmatonline.phmsa.dot.gov/services/publication_documents/Transporting%20Infectious%20Substances%20Safely.pdf