



## Zika Virus Specimen Submission Instructions for RT-PCR and IgM Serology

### 1. Criteria for Zika Virus Testing Approval

- **Patients must meet epidemiologic testing criteria prior to approval for testing.**
  - To discuss testing, clinicians within Tarrant County should contact the TCPH Epidemiology Division at 817-321-5350 and complete the required TCPH Zika Virus Specimen Submission Form.
  - Clinicians outside of Tarrant County should contact either their respective county health department or the Texas PHR 2/3 Zoonosis program at 817-264-7920 or 817-822-6786 (after business hours) and complete the required TCPH Zika Virus Specimen Submission Form. If a sample needs to be submitted after business hours, on weekends, or holidays, call 817-475-3093.
- **Criteria for Testing at TCPH Include**
  - a) Any patient (including pregnant female) with **1 or more major symptoms** compatible with Zika virus infection (i.e. fever, rash, joint pain/myalgia, or conjunctivitis), who also meet at least one of the following criteria:
    - 1) Onset **during or within 2 weeks of travel** to an area of active Zika transmission
    - 2) Onset **within 2 weeks of sexual exposure** to a partner with possible Zika exposure
  - b) Asymptomatic, pregnant female, and at least one of the following criteria within the previous 12 weeks:
    - 1) History of travel to an area of active Zika transmission
    - 2) History of sexual exposure to a traveler to an area of active Zika transmission
  - c) Infant whose mother had Zika exposure during pregnancy, if:
    - 1) Evidence of abnormalities consistent with Zika exposure or adverse birth outcomes
    - 2) No evidence of abnormalities consistent with Zika, but mother has laboratory evidence of Zika infection
- **RT-PCR Testing**
  - To diagnose Zika virus, always collect serum. In addition, it is recommended to also collect urine, which increases the probability of detection of Zika virus in a patient with Zika disease. The likelihood of detection is highest if the serum and urine samples are collected during the acute period of the disease (<14 days after symptom onset). If the patient makes the first visit to the physician ≥14 days after onset of symptoms, serum may still be collected for serology, but an Epidemiologist at your local health department will determine on a case-by-case basis whether RT-PCR testing should also be performed.
  - In addition, cerebrospinal fluid (CSF) and amniotic fluid are approved specimen types for Zika RT-PCR. Please note, a serum sample must also be submitted when a CSF or amniotic fluid specimen is submitted. The serum specimen may be tested by RT-PCR and/or serology, as appropriate.
- **Serological (IgM) Testing**
  - To test for anti-Zika virus IgM antibodies, collect a serum or plasma sample. Serum is the preferred specimen type. If plasma is collected, it **MUST** still be accompanied by a serum specimen for possible confirmatory testing. In order for the specimen to be tested for IgM antibodies, samples must be collected 8 days or more after onset of symptoms and no later than 12 weeks after the infection is thought to have occurred, as anti-Zika IgM antibody levels may decline.
  - Negative (non-reactive) results reported on specimens that were potentially collected before 8 days after onset of symptoms (e.g., in the event it is uncertain what day symptoms began or exposure occurred) **MUST** be retested with a new sample collected at least 7 days from the first specimen. This will reduce possibility of false-negative IgM results.
- When more than one specimen type is submitted, ensure that the tubes are labeled in a manner that will allow NTRL to differentiate between each type of specimen.

### 2. Specimen Collection and Types Accepted

#### **Serum (required for RT-PCR testing):**

- At least 2 mL of **serum** is required for Zika RT-PCR testing. Whole blood will not be accepted.
- Collect at least 5 mL blood in a serum separator tube (e.g., gold top, tiger top). Centrifuge to separate the serum from the red blood cells. Transfer the serum into a transport tube with a screw cap. **DO NOT freeze** the serum separator tube for shipping. Freezing can cause hemolysis, and hemolyzed specimens will be unsatisfactory for testing.



**Plasma (acceptable for IgM testing, but a serum specimen MUST also be submitted):**

- At least 2 mL of **plasma** is acceptable for Zika IgM testing. Whole blood will not be accepted. Plasma cannot be used for RT-PCR testing or for confirmatory testing (e.g., PRNT testing) that may be necessary to confirm presumptive positive IgM results.
- Collect at least 5 mL blood in a EDTA tube (e.g., purple top). Centrifuge to separate the plasma from the red blood cells. Transfer the plasma into a transport tube with a screw cap. **DO NOT freeze** the EDTA tube for shipping. Freezing can cause hemolysis, and hemolyzed specimens will be unsatisfactory for testing.

**Urine, CSF, Amniotic Fluid**

- At least 1 mL of **urine, cerebrospinal fluid (CSF), or amniotic fluid** is required.
- Collect urine, CSF, or amniotic fluid without any additives.
- Transfer urine to a tube with a tight-sealing lid – **DO NOT** submit urine collection cups.
- Transfer any other body fluid (CSF, amniotic fluid) to a tube with tight-sealing lid or screw cap.
- Clearly label each tube so that specimen types can be differentiated.

**3. Criteria for Specimen Handling**

- All specimen containers **MUST** be labeled with the patient's first name, patient's last name, and patient's date of birth, specimen collection date, and specimen collection time.
- If multiple specimen types are collected, clearly label each one so that they can be identified.
- All specimens must be placed in a biohazard bag and must be accompanied by a completed printed copy of the TCPH Zika Virus Specimen Submission Form for RT-PCR and IgM Serology. Keep the submission form separated from the primary specimen container.
- Specimens must be placed in a biohazard bag and stored at 2-8°C or frozen as indicated below:
  - Specimens that are shipped the same day of collection and will arrive at the lab within 48 hours of collection can be stored at 2-8°C and must be shipped with cold packs.
  - Specimens that will be stored and arrive at the lab more than 48 hours after collection must be stored frozen and shipped on dry ice.

**4. Specimen Transport**

- **Approval of the TCPH Epidemiology division for facilities within Tarrant County is required before a specimen can be sent to the Tarrant County Public Health LRN Laboratory for testing.**
- **Facilities outside of Tarrant County must contact their respective county or regional health department for case reporting and approval prior to specimen submission to the TCPH LRN Laboratory.**
- Submitters are responsible for arranging transport of specimens from their facility to TCPH.
- Refrigerate urine, CSF, amniotic fluid and/or serum or plasma samples at 2-8°C and ship on cold packs within 48 hours of collection. Specimens that will arrive at the lab more than 48 hours after collection must be stored frozen and shipped on dry ice.
- Follow packing and shipping instructions for Category B, Biological Substances.
- Ship according to IATA (International Air Transport Association) guidelines using overnight courier to arrive during business hours Monday-Friday, 8:00 AM-4:00 PM.
- Specimens delivered by same-day courier services will also be accepted.

**5. All sections of the submission form **MUST** be completed in their entirety.**

**6. The TCPH LRN Laboratory can only accept specimens from facilities within the following counties comprising its service area:** Archer, Baylor, Brown, Callahan, Clay, Comanche, Coleman, Cooke, Denton, Eastland, Erath, Foard, Hardeman, Haskell, Hood, Jack, Johnson, Jones, Knox, Montague, Palo Pinto, Parker, Runnels, Shackelford, Somervell, Stephens, Tarrant, Taylor, Throckmorton, Wichita, Wilbarger, Wise, and Young.

**TCPH LRN Shipping and Delivery Address:**

Tarrant County Public Health  
North Texas Regional Laboratory  
Bioterrorism and Emerging Agents Section  
1101 South Main Street  
Fort Worth, Texas 76104

**Contact Phone Numbers:**

North Texas Regional Laboratory: 817-321-4774  
Tarrant County Epidemiology: 817-321-5350  
TX DSHS HSR 2/3 Epidemiology: 817-264-4541



**Tarrant County Public Health**  
**North Texas Regional Laboratory**  
 Bioterrorism Response/Emerging Agents Section  
 1101 South Main Street, Fort Worth, TX 76104  
 Phone: 817-321-4774  
 CLIA# 45D0659873

For TCPH Use Only:

TCPH Lab ID:	
Epi Case #:	
Condition at receipt:	<input type="checkbox"/> Frozen <input type="checkbox"/> Refrigerated <input type="checkbox"/> Ambient <input type="checkbox"/> Appropriate for testing <input type="checkbox"/> Rejected
Received by:	
Date/Time Received:	

**Zika Virus Specimen Submission Form for RT-PCR and IgM Serology**

Note: RT-PCR, serology or both will be performed at NTRL and the testing process is determined by clinical symptoms and epidemiological criteria. Submissions will also be tested for Chikungunya and Dengue viruses by RT-PCR, as appropriate.

All Patient, Submitter, and Specimen Information Sections MUST be completed in their entirety for specimen acceptance

Patient

Last Name: \_\_\_\_\_ First Name: \_\_\_\_\_ Patient ID/ Medical Record #: \_\_\_\_\_  
 Date of Birth: \_\_\_\_\_ Sex:  Male  Female Race/Ethnicity: \_\_\_\_\_  
 Address: \_\_\_\_\_ Street Address or PO Box City State Zip Code  
 County: \_\_\_\_\_ Phone No: \_\_\_\_\_ Alternate Phone No: \_\_\_\_\_

Submitter

Hospital/Lab/Clinic Name: \_\_\_\_\_  
 Physician Name: \_\_\_\_\_  
 Address: \_\_\_\_\_ Street Address or PO Box City State Zip Code  
 Contact Name: \_\_\_\_\_ Phone No: \_\_\_\_\_ Fax No: \_\_\_\_\_  
 County: \_\_\_\_\_ Name of Person Completing This Form: \_\_\_\_\_

Specimen(s) & Requested Test(s)

**Important Specimen Collection Instructions:** Collect serum specimen in a SST blood tube (e.g. gold or tiger top) OR plasma specimen in an EDTA tube (e.g. purple top). Specimen(s) MUST be centrifuged and serum or plasma separated from red cells. Refrigerate (2-8°C) all sample types and deliver to lab on cold packs within 48 hours of collection. Specimens that will arrive at the lab more than 48 hours after collection must be stored frozen and shipped on dry ice. Specimens that do not meet these submission requirements cannot be tested.

To diagnose Zika by RT-PCR, the laboratory requires serum and urine samples collected <14 days after symptom onset. Serum must always be submitted, even if urine, CSF, or amniotic fluid is submitted. Please indicate specimen type on each tube or vial.

RT-PCR (appropriate for serum, urine, amniotic fluid and CSF specimens collected <14 days after symptom onset)  
 Zika IgM Serology (appropriate for serum or plasma specimens collected at 8 days or more after symptom onset)  
 Zika IgM Serology repeat (appropriate for serum or plasma specimens that may have been collected less than 8 days after symptom onset and were recommended to be re-tested with a new sample taken at least 7 days after first tested sample)

**Required Specimen:**  Serum  
**Additional Specimen(s):**  Plasma (Zika IgM testing only)  
 Urine (Zika PCR testing only)  
 Amniotic Fluid (Zika PCR testing only)  
 CSF

Collection Date (MM/DD/YYYY) \_\_\_\_\_ Collection Time (Hour:Min; Indicate AM or PM) \_\_\_\_\_

Storage Condition of Specimen(s) Prior to Shipment:  Refrigerated (2-8°C)  Frozen (-20°C or colder)

Epidemiology

Flavivirus Exposure History		Yes	No	Unk	Date	Symptoms (check all that apply)
Yellow Fever	Vaccination					
	Disease					
Japanese Encephalitis	Vaccination					
	Disease					
Tick-borne Encephalitis	Vaccination					
	Disease					

Is patient pregnant?  No  Yes; # of weeks gestation: \_\_\_\_\_

Did the patient travel to an area with Zika transmission<sup>†</sup> within 14 days prior to symptom onset?  Unknown  No  Yes  
 If yes, list all countries/cities/dates of travel: \_\_\_\_\_

Does the patient's sexual partner have a history of illness consistent with Zika virus disease and a history of travel to an area with Zika transmissions?  Unknown  No  Yes  
 If yes, date of symptom onset: \_\_\_\_\_ AND list all countries/cities/dates of travel: \_\_\_\_\_

† See [cdc.gov/zika/geo/index.html](http://cdc.gov/zika/geo/index.html) for current list