



Zika Sample Collection Guidance

General information:

This document provides sample collection instructions for Zika Virus testing. For any questions regarding specimen collection or shipment for Zika testing, please contact the DC Public Health Laboratory at Zikalab@dc.gov.

Serum specimen:

- Collect two tubes of serum (containing NO anticoagulant or preservative) with at least 6 milliliters (mL) of blood per tube (e.g., red top, tiger top, speckle top, gold top, or other serum separator tube). Do NOT use any blood collection tubes containing anticoagulants or preservatives (e.g., green top, yellow top, or purple top).
- After collection of whole blood allow the blood to clot by leaving it undisturbed in the laminar flow hood for 15 – 30 minutes, but not longer than 1 hour. NOTE: Please be sure to follow blood collection tube manufacturer's instructions on how to properly allow for clot formation.
- Promptly (within 1 - 6 hours) centrifuge serum according to manufacturer's instructions. Use of serum separator tubes is recommended.
- Using sterile laboratory technique, transfer the serum into a sterile tube using a sterile transfer pipette. The sterile tube should then be sealed in paraffin wax to prevent spillage. The transfer pipette should be discarded in a biohazard bag.
- Label the tube with the patient's name (last, first), date of birth, Zika ID, and date and time of blood draw, and specimen type (serum, urine).
- Serum samples should be placed in an ultra-low temperature freezer and maintained at -70°C while awaiting sample pick-up by the DC-PHL courier.
- Specimens that leak will not be tested.

Urine specimen:

- A urine and serum sample needs to be collected simultaneously and sent for testing.
- Collect 5 - 20 mL of urine in a sterile screw-top container. The sterile container should then be sealed in paraffin wax to prevent spillage.
- Label the tube with the patient's name (last, first), date of birth, Zika ID, date and time of blood draw, and specimen type (serum, urine).
- Place the sealed urine container in a specimen transport bag; seal the bag. Then place the sealed transport bag into a second specimen transport bag with the same patient's serum specimens. Seal the second (outer) bag containing both urine and serum specimens. Place the test requisition form in the sleeve of the outer bag.



- Urine specimens should be kept frozen at -70°C together along with the same patient's serum specimen while awaiting sample pick-up by the DC-PHL courier.
- Specimens that leak will not be tested.

Cerebrospinal fluid (CSF) specimen:

- All CSF samples must be accompanied by a serum sample to be eligible for testing.
- Collect CSF within 7 days of symptom onset.
- It is recommended that 3 mL of CSF be collected, however at least 1 mL CSF is required for testing. Only tubes 2 - 4 should be sent due to the potential of blood and sterility of the initial CSF tube.
- Label the tube with the patient's name (last, first), date of birth, Zika ID, date and time of blood draw, and specimen type (serum, urine).
- Seal the sterile tube with paraffin wax and place in specimen biohazard bag with absorbent material.
- For antibody and rRT-PCR testing, specimens should be kept cold ($2-8^{\circ}\text{C}$).

Other body fluid specimen:

Amniotic fluid:

- Collect 3 - 5 mL of amniotic fluid in a sterile screw-top container (15 mL centrifuge tube). Use paraffin wax to seal.
- Label the tube with the patient's name (last, first), date of birth, Zika ID, date and time of blood draw, and specimen type (serum, urine, amniotic fluid).
- Place the tube in specimen collection bag with absorbent material.
- Amniotic fluid specimens should be kept frozen (-70°C) for storage and shipping.

Tissue Specimens (placental tissue, umbilical cord tissue)

- For placenta and fetal membranes, several full thickness pieces including **at least 3** full thickness ($0.5-1\text{cm} \times 3-4\text{cm}$ in depth) from middle third of placenta disk AND **at least 1** from the placenta disk margin and one 5×12 cm strip of fetal membrane should be sent.
- All tissues need to be fixed in formalin. The volume of formalin used to fix tissues should be 10x the volume of tissue. Place tissue in 10% buffered formalin for a minimum of three days or until fully fixed. After fixation, tissue can be transferred to 70% ethanol for long term storage.
- If stored prior to shipping, please transfer fixed tissues to 70% ethanol after 72 hours.
- Fixed tissues should be stored and shipped at **room temperature** (ambient).
- Paraffin blocks should be submitted in accordance with these instructions for formalin-fixed specimens.



- **DO NOT FREEZE samples that have been fixed in formalin.**
- For further instructions on sending tissue samples, please refer to <http://www.cdc.gov/zika/laboratories/test-specimens-tissues.html>.

Specimen labelling:

Please be sure to properly label **ALL** specimens: Failure to properly label a specimen will result in rejection and the specimen will not be tested. Specimens **must** be labeled with:

- Patient's first and last name
- Patient's date of birth
- Zika ID
- Date and time of collection
- Specimen type (e.g., serum, urine, CSF)

All information on the specimen labels must **exactly** match the information on the PHL test request form, including the spelling of the patient's first and last names and date and time of collection. Specimens received with discrepant information on the labels and requisition forms will not be tested.

Specimen storage and shipping:

- All specimens need to be stored at -70°C if not picked up within 72 hours (e.g., sample collection was completed on a Friday afternoon and pick-up will not occur until Monday).
- **Note:** It is important that frozen samples not thaw during shipping. To prevent this be sure to ship with dry ice and follow proper Department of Transportation (DOT) and International Air Transport Association (IATA) shipping regulations.

Additional Testing:

- Positive (reactive) IgM results by ELISA should be confirmed by testing for neutralizing antibodies. Plaque-reduction neutralization tests (PRNTs) can be performed to measure virus-specific neutralizing antibodies and may be able to discriminate between cross-reacting antibodies in primary flavivirus infections.
- The DC PHL does not perform PRNT and will forward all approved specimens to the Centers for Disease Control and Prevention (CDC) for confirmatory PRNT testing.

REQUIRED DOCUMENTATION FOR TESTING AT THE DC PHL

- DC Public Health Laboratory Test requisition form
- DC Public Health Laboratory Chain of Custody form

GOVERNMENT OF THE DISTRICT OF COLUMBIA

SCIENCES

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Note: if the proper paperwork is not complete upon pick-up, the courier has the right to refuse the sample. Please do not email for pick-up until all forms are complete.

For questions regarding sample collection, storage, packaging, and shipping, please contact the District of Columbia Public Health Laboratory at (202) 481-3419.