California Department of Public Health
Viral and Rickettsial Disease Laboratory

LABORATORY TESTING GUIDANCE FOR ARBOVIRUSES

Appropriate Clinical Specimens for Chikungunya, Dengue, and Zika Virus Testing

Virus	Testing Method	Serum	CSF	Whole Blood	Urine/Amniotic Fluid
Chikungunya virus	Real-time RT-PCR	Yes	Yes	Yes	No
	IgG & IgM Serology	Yes	No	No	No
	Neutralizing Ab Serology	Yes[1]	Yes[1]	No	No
Dengue virus	Real-time RT-PCR	Yes	Yes	Yes	No
	IgG & IgM Serology	Yes	No	No	No
	Neutralizing Ab Serology	Yes	Yes[1]	No	No
Zika virus	Real-time RT-PCR	Yes	Yes	Yes	Yes
	IgM Serology	Yes	No	No	No
	Neutralizing Ab Serology	Yes	Yes[1]	No	No

[1] *For Surveillance Use Only*

Required Forms

- Complete the VRDL General Purpose Specimen Submittal Form for **EACH SPECIMEN** (VRDL Specimen Submittal Forms):
  - Provide onset date, travel history, symptoms; for Zika testing please provide pregnancy status, estimated delivery date, and fetal ultrasounds findings if performed.
  - Include any previous arbovirus test results.

Specimen Collection

- **CSF or EDTA whole blood MUST BE accompanied by a serum sample**
- Serum: 3-5 ml of whole blood in a red top or serum separator tube, **OR**
  1 ml serum in a sterile collection tube.
- Whole blood: 3-5 ml in EDTA (lavender/purple top) tubes.
- CSF: collect 1 ml CSF in a sterile collection tube.
- Urine: 10-40 ml in sterile collection tubes. Urine must be submitted with an accompanying serum.
  - Note: Collected as close to onset as possible and no longer than 21 days after onset.
- Amniotic Fluid: 1 ml in sterile collection tubes.

Specimen Storage and Shipping

- Store all specimens at 4°C and ship on cold packs if shipped to VRDL within 72 hours from collection.
- Freeze processed specimens at -20oC and ship on dry ice if shipped to VRDL after 72 hours from collection. Do NOT freeze whole blood. Do NOT freeze urine unprocessed.
- Ship approved specimens to:

CDPH VRDL
Attn: Specimen Receiving
850 Marina Bay Parkway
Richmond, CA 94804

Interpreting Arboviral Laboratory Results

- **Arboviral serologic assays:** Assays for the detection of IgM and IgG antibodies commonly include enzyme-linked immunosorbent assays (EIA or ELISA) and immunofluorescence assays (IFA). These assays provide a presumptive diagnosis, with the caveat that serologic detections may be due to cross-reactivity among the closely related arboviruses. Definitive diagnosis should include confirmatory testing. Confirmatory testing may involve the detection of arboviral-specific neutralizing antibodies using assays such as plaque reduction neutralization test (PRNT).
- **Serologic cross-reactivity:** In some instances, arboviruses from the same genus produce cross-reactive antibodies. In geographic areas where two or more closely-related arboviruses occur, serologic testing for more than one virus may be needed and results compared to determine the specific causative virus. For example, such testing might be needed to distinguish antibodies resulting from infections within genera, e.g., flaviviruses such as West Nile, St. Louis encephalitis, Powassan, Dengue, or Japanese encephalitis viruses.
- **Rise and fall of IgM antibodies**: For most arboviral infections, IgM antibodies are generally first detectable at 3 to 8 days after onset of illness and persist for 30 to 90 days, but longer persistence has been documented (e.g., up to 500 days for West Nile virus). Serum collected within 8 days of illness onset may not have detectable IgM and testing should be repeated on a convalescent-phase sample to rule out arboviral infection in those with a compatible clinical syndrome.
- **Persistence of IgM antibodies:** Arboviral IgM antibodies may be detected in some patient's months or years after their acute infection. Therefore, the presence of these virus-specific IgM antibodies may signify a past infection and be unrelated to the current acute illness. Finding virus-specific IgM antibodies in CSF or a fourfold or greater change in virus-specific antibody titers between acute- and convalescent-phase serum specimens provides additional laboratory evidence that the arbovirus was the likely cause of the patient's recent illness. Clinical and epidemiologic history also should be carefully considered.
- **Persistence of IgG and neutralizing antibodies:** Arboviral IgG and neutralizing antibodies can persist for many years following a symptomatic or asymptomatic infection. Therefore, the presence of these antibodies alone is only evidence of previous infection and clinically compatible cases with the presence of IgG, but not IgM, should be evaluated for other etiologic agents.

Additional Resources

- VRDL Zika Virus Information: https://www.cdph.ca.gov/Programs/CID/DCDC/Pages/Zika_VRDL.aspx
- VRDL Website: https://www.cdph.ca.gov/Programs/CID/DCDC/Pages/VRDL.aspx
- For questions about specimen collection, submittal, or shipping, please contact the VRDL Medical and Epidemiology Liaison Section at (510) 307-8585.