Laboratory Preparedness and Response Branch
Biological Response Section

Specimen Requirements for Zika, dengue, Chikungunya (Trioplex) multiplex Real-Time (rti) Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR)

Methodology:	CDC Trioplex Real-Time RT-PCR Assay
Performed:	The Trioplex real-time (TaqMan®) RT-PCR assay is for detection and differentiation of RNA from dengue, chikungunya and Zika viruses in serum, cerebrospinal fluid (CSF), and for the detection of Zika virus RNA in urine and amniotic fluid. This protocol has been designed to facilitate simultaneous testing for the presence of dengue, chikungunya and Zika viruses using a single sample. Viral RNA is generally detectable in serum or urine during the acute phase of infection. Positive results are indicative of current infection. The assay is approved for diagnostic use under a U.S. Food and Drug Administration Emergency Use Authorization (EUA).
Criteria for testing:	Department of Health (DOH) approval by the Disease Investigation Branch (DIB), Disease Outbreak and Control Division, is **required** before specimens will be tested.

See DOH Medical Advisory of 8/2/2016

Pregnant women with possible Zika exposure:
- Symptomatic <2 weeks: urine & serum PCR (negative reflex to serum IgM for Zika & dengue)
- Symptomatic 2-12 weeks: urine & serum (positive /equivocal serum IgM; reflex to urine & serum PCR)
- Asymptomatic <2 weeks after exposure: urine & serum PCR (if negative, return 2-12 weeks for serum IgM)
- Asymptomatic 2-12 weeks urine & serum (positive /equivocal serum IgM; reflex to urine & serum PCR)
- Any >12 weeks: urine & serum; test based on condition (see Medical Advisory)
- Any amniotic fluid with urine & serum (performed at CDC)
- Tissue specimens (see submission procedures on CDC website)

Non-pregnant patients with possible Zika exposure:
- Symptomatic <2 weeks: urine & serum PCR (negative reflex to serum IgM)
- Symptomatic 2-12 weeks: serum IgM for Zika & dengue (positive /equivocal **may** reflex to serum PRNT; see MMWR of 7/26/2016)

	Patients with neurological symptoms & possible Zika exposure: - Neurological symptoms <7 days: CSF, urine, & serum PCR - Neurological symptoms >7 days: CSF, urine, & serum; CSF or serum IgM **may** reflex to PCR
Turn-Around-Time:	Results are reported 2-3 business days after approval and receipt of specimen(s). For Specimens submitted by the USAPI: Results for outbreak related specimens are reported 3-4 business days from receipt of suitable specimen(s). Results for surveillance samples are reported 5-7 business days from receipt of suitable specimen(s).
Specimen type required:	Venous blood sample: Follow device manufacturer's instructions for proper serum collection and separation. Whole blood (EDTA) Urine (**Zika only**): Submit an aliquot in a small, sterile, screw cap micro tube. Do not submit in urine collection cups. Urine must be submitted with a patient-matched serum specimen. A minimum of 1.5 ml serum AND urine is required. CSF: A minimum of 1.5 ml WITH serum AND urine. Test performed at CDC. **CSF can only be tested when submitted with patient-matched serum & urine.** Amniotic fluid (**Zika only**): A minimum of 0.5 ml WITH serum AND urine. Test performed at CDC. Tissue: Test performed at CDC; see submission instructions on CDC website. **Required information:** 1. Date of onset of symptoms 2. Date of specimen collection 3. Any **pertinent travel history** (3 months prior)
Specimen storage/transport:	Refrigerate specimens at 4°C or maintain on ice for no longer than 24 hours. If storage/transport will exceed 24 hours, freeze serum at -20°C or lower.

	Ship separated specimens on cold packs (i.e. 4˚C) within 24 hours. Ship on dry ice if the specimens have been frozen at -20° C or lower. Follow packing and shipping instructions of the U.S. Department of Transportation (U.S. DOT), and the International Air Transport Association (IATA) for Biological Substance, Category B.
Specimen submission:	Submitters: Clinical laboratories and the DIB. Please notify DIB and the State Laboratory Division (SLD) Biological Response Section prior to the submission of specimens.
Criteria for rejection:	**Specimen without prior approval from the State Epidemiologist or DIB Epidemiologist Investigator.** • Specimen is received in a container that is leaking. Specimen **will not be processed** if the safety of the laboratory worker is compromised. • Specimen is not collected in a proper container (e.g., heparin tube) or handling instructions are not followed, which compromise test quality. Submitter will be asked to submit another specimen. • Specimen quantity is not sufficient (QNS) to perform the tests. Submitters will be notified to submit another specimen. • Specimen is not received at 4°C or packed in blue ice; • Frozen specimens not shipped in dry ice. • Incomplete submission (e.g., urine without serum). • Unlabeled or incomplete specimen labeling and documentation; Submitter will be notified to provide correct information and corrected SLD Form 81.3. • Specimen label does not match the requisition.
Stability:	Refrigerate at 2-8˚C for no longer than 24 hours. If the specimen cannot be transported to the SLD within 24 hours after collection and separation, it should be frozen at -20˚C or lower. Frozen samples must be shipped in dry ice if possible.
Requisition Form:	Each specimen submitted must have a completed SLD Form 81.3. Submitter is responsible for completing SLD Form 81.3 including but not limited to the following information: at least two patient unique identifiers, date of onset of illness, signs and symptoms, travel history,

	immunization history, test(s) requested, name and address of submitter. Requisition forms shall be placed in a separate bag and shall not be packed with the specimen(s).
Normal Value (Serum and CSF):	No Zika, dengue, or chikungunya RNA detected by rRT-PCR.
Normal Value (Urine: and amniotic fluid)	No Zika RNA detected by rRT-PCR.
Result Notification:	Laboratory reports are electronically relayed to the submitters via the SLD dashboard. An automatic notification will be relayed when a report is available on the SLD dashboard. Laboratories who do not receive automatic notification will be notified by e-mail on the availability of results on the SLD Dashboard. Submitters who do not have access to electronic reports will be notified via fax or by encrypted e-mail.
Test performed at:	Biological Response Section (BRS) Laboratory Preparedness and Response Branch (LPRB) State Laboratories Division Department of Health 2725 Waimano Home Road Pearl City, Hawaii 96782
Contact:	Remedios Gose at (808) 453-5993 or (808) 554-9992 or Cheryl Daquip at (808) 453-5984 or (808) 453-6641

Reviewed By:

Remedios B. Gose — 12/4/19

Remedios B. Gose, MSPH, RM (NRCM) — Date
Laboratory Preparedness and Response Branch Chief

Approved By:

Edward P. Desmond — 12/4/2019

Edward Desmond, Ph.D. — Date
Administrator, State Laboratories Division