GUAM PUBLIC HEALTH LABORATORY GUIDELINES
SPECIMEN REQUIREMENTS FOR ZIKA VIRUS
IN CLINICAL SAMPLES

Methodology:	**1. CDC Zika Virus Real-time (rti) Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) Assay** **2. CDC Zika Virus IgM Antibody Capture Enzyme Linked Immunosorbent Assay (MAC-ELISA)** **3. CDC Trioplex Real-time (TaqMan®) Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) Assay**
Specimen Submission Guidelines	• Call the DPHSS at 671-689-3942 for pre-approval, before collecting and sending specimens for suspected Zika Virus. **NO SPECIMEN WILL BE ACCEPTED WITHOUT PRIOR CONSULTATION.** • Once vetted/approved by DPHSS, complete the specimen submission form with patient's name (last name, first name), date of birth, unique Identification (ID) number/specimen ID number, date of collection, time of collection, initial of collector (Note: Test subject to CLIA regulations and these requirements). • Specimens for transport to the GPHL must be placed in a sealed biohazard transport bag and placed in a sealed, decontaminated primary sterile container. • Call the GPHL (671) 300-9082 or (671) 300-9085 to inform staff of the specimen delivery. • Physicians/providers will be notified of the result by the GPHL Technologist once completed.
Performed at Hawaii State Lab:	1. CDC Zika Virus Real-time RT-PCR Assay is a **non-FDA** approved assay developed by the CDC • For the detection of the precursor membrane proteins/membrane proteins (prM/M) and the envelope proteins during maturation of the Zika virus. • In which a Confirmatory testing of an equivocal result is performed at the CDC. 2. CDC Zika Virus IgM Antibody Capture Enzyme Linked Immunosorbent Assay is an **FDA authorized** assay, issuing the Emergency Use Authorization (EUA) to test specimens according to the CDC criteria • An *in vitro* diagnostic test for the detection of the Zika virus and Zika virus infection. This EUA will terminate when the Human Health and Services (HHS) Secretary's declaration terminates, unless FDA revokes it sooner. • In which a Confirmatory testing of an equivocal result is performed at the CDC.

Guam Public Health Lab:	3. CDC Trioplex Real-time RT-PCR Assay is an **FDA authorized** assay, issuing the Emergency Use Authorization (EUA) to test specimens according to the CDC criteria • For the detection and differentiation of RNA from dengue, chikungunya and Zika viruses in serum and for the detection of Zika virus RNA in urine. • In which a Confirmatory testing of an equivocal result is performed at the CDC.
Criteria for PCR testing:	Testing of clinical blood specimens (serum) with the CDC Zika Virus Real-Time RT-PCR Assay or testing of clinical blood specimens (serum) and urine specimens with the CDC Trioplex Real-time RT-PCR Assay **should not be performed** unless the patient meets clinical signs and/or symptoms compatible with Zika virus infection and/or specimens meeting the case definition set and/or updated by the CDC and/or the Bureau of Communicable Disease Control (BCDC) under the Division of Public Health of the Department of Public Health and Social Services (DPHSS). Clinical Findings are characteristically acute onset of fever with maculopapular rash, arthralgia, or conjunctivitis. Other commonly reported symptoms include myalgia and headache. Clinical illness is usually mild with symptoms lasting for several days to a week. **Note**: **Negative results obtained with this test do not preclude the diagnosis of Zika virus and should not be used as the sole basis for treatment or other patient management decisions.**
Criteria for MAC ELISA testing:	Testing of clinical blood specimens (serum) or cerebral spinal fluid (CSF) with the CDC Zika Virus MAC-ELISA **should not be performed** unless the patient meets testing criteria and/or post travel or exposure compatible with Zika virus infection and/or specimens meeting the case definition set and/or updated by the CDC and/or the Bureau of Communicable Disease Control (BCDC) under the Division of Public Health of the Department of Public Health and Social Services (DPHSS). **Note**: Negative results obtained with this test do not preclude the diagnosis of Zika virus and should not be used as the sole basis for treatment or other patient management decisions.
For private clinics and providers:	**Specimen Submission Guidelines** **Note: All requests require BCDC approval. Do not send specimens before consulting with BCDC. Any requests for testing will be referred to BCDC for review. Only those specimens approved for testing by the DPHSS will be tested.**

For private clinics and providers (cont.):	1. Submit minimum of 1ml serum collected in red top or in separator tube (marble or tiger-top). Other acceptable specimen: 1 ml cerebral spinal fluid (CSF) in sterile container, but CSF will be tested for ZIKA MAC-ELISA only, OR 1 ml urine in sterile container, but urine will be tested in conjunction with serum for ZIKA Trioplex rRT-PCR only. 2. **Fill out required form(s) COMPLETELY.** Include the following information date of onset of illness, signs and symptoms and travel history. Zika specimens **must be collected** within fourteen (14) days from onset of signs and symptoms OR between two (2) to twelve (12) weeks post travel or exposure for asymptomatic pregnant females. Send forms with the specimen. 3. Refrigerate specimens at 4°C or maintain on ice for no longer than 72 hours. If storage/transport will exceed 72 hours, freeze serum at -20°C or lower. 4. Send specimens on ice to GPHL business day Mondays-Fridays 8AM-430PM.
Specimen Required:	The laboratory requires a blood sample in conjunction with urine sample taken during the acute period of the disease up to 14 days from onset of the symptoms. **Note: If the patient makes the first visit to the physician on or after day 7 of onset of the symptoms, blood sample collected is likely not to render a positive RT-PCR result. For asymptomatic pregnant females, the laboratory requires a blood sample taken during the first trimester (2-12 weeks) post travel or exposure.**
Specimen Collection:	• Once there is a clinical diagnosis of suspected Zika virus, take a venous, whole blood sample, and collect a sterile container of urine sample. • Follow serum and urine specimen collection devices manufacturer instructions for proper collection, separation and storage methods. Separated serum samples must be maintained on ice or in a refrigerator before it is sent to GPHL.
Specimen Transport, Storage and Stability:	Store and transport specimens on ice. For USAPI Laboratories, follow the PIHOA Shipping Mechanism Guidelines.
Specimen Submission:	• Ensure that when transporting human blood, plasma or serum specimens, urine specimens or cerebral spinal fluid specimens all applicable regulations for transport of potentially infectious biological specimens are met. • Transport/ship human serum or plasma or urine or cerebral spinal fluid samples on ice. Follow instructions for Class B – Biological Substance of the U.S. Department of Transportation (U.S. DOT) and International Air Transport Association (IATA) for packing and shipping.

	The submitting facility must notify BT Microbiologist or alternate of GPHL at (671) 300-9080/9083/9082 prior to submitting/ shipping specimens. **NOTE: It is the responsibility of the submitter to track the arrival of the specimens along with the Zika Specimen form at GPHL to ensure that these specimens are received by the Laboratory staff.**
Rejection Criteria: **Rejection Criteria (cont.):**	• Specimens not meeting criteria for testing; • Specimen is not collected in a proper container or special handling instruction is not followed; • Specimen quantity is insufficient to perform the test; • Specimen received in a container that is leaking; • Specimen is not received at 4°C or packed in ice pack; • Blood collected with heparin or EDTA tube; • Unlabeled specimens; • Illegible/ incomplete labeling/documentation.
Submission Form:	Specimen Laboratory Submission Form • Each specimen submitted must have a completed Guam Public Health Laboratory Submission Form GPHL DPHSS_FRM_05/18/16, with the patient name, patient identification number, type of specimen, date/time of collection, submitter, date of onset, travel history, date shipped/sent to GPHL, test(s) requested and other pertinent information. • Submission forms that are not consistent with the specimen submitted will be rejected and requesting facility will be asked to re-submit. • Submission forms must not be in direct contact with the specimen(s). • Fill out required form(s) **COMPLETELY.** >Incomplete forms will be rejected.
Result Notification:	Laboratory reports will be forwarded by GPHL, to the submitting facility and the BCDC Administrator via Facsimile. Any other request for copies of laboratory reports, apart from that stipulated above will not be accepted.
Contact:	Alan Mallari, Microbiologist III, GPHL (671) 300-9080 or (671) 300-9081 alanjohn.mallari@dphss.guam.gov Lea Nisay, Microbiologist II, GPHL(Alternate) (671) 300-9083 (671) 300-9098 FAX lea.nisay@dphss.guam.gov

	Anne Marie Santos, Central Laboratory Administrator, GPHL (671) 300-9082 or (671) 300-9085 annemarie.santos@dphss.guam.gov

Reference:

1. CDC Zika Virus Real Time RT-PCR Assay LPRP, BRS SOP
2. CDC Zika Clinical Evaluation and Disease; http://www.cdc.gov/zika/hc-providers
3. CDC Zika Reports in MMWR; http://www.cdc.gov/mmwr/index.html