Laboratory Preparedness and Response Branch
Biological Response Section

Specimen Requirements for Ebola Real-Time Reverse-Transcriptase Polymerase Chain Reaction (rRT-PCR)
For Use Under an Emergency Use Authorization (EUA) Only

Methodology:	Ebola Virus Real-Time RT-PCR Assay (CDC)
Performed:	CDC's Ebola Virus real-time Reverse-Transcriptase Polymerase Chain Reaction (rRT-PCR) Assay is an FDA-approved Emergency Use Authorization (EUA) assay for the in vitro qualitative detection of Ebola virus RNA in clinical specimens from individuals meeting Ebola virus clinical and/or epidemiological criteria (clinical signs and symptoms associated with Ebola, contact with a probable or confirmed Ebola virus case, history of travel to geographic locations where Ebola virus cases were detected, or other epidemiologic links for which Ebola virus testing may be indicated as part of a public health investigation).
Criteria for testing:	**Mandatory consultation required.** Consultation with and authorization from the Department of Health (DOH), State Epidemiologist is required **PRIOR** to collecting and submitting specimens to the State Laboratories Division (SLD) for testing. The DOH will coordinate with Centers for Disease Control and Prevention (CDC). This test should only be performed for individuals who have both consistent signs or symptoms and risk factors such as: 1. Elevated body temperature or subjective fever or symptoms, including severe headache, fatigue, muscle pain, vomiting, diarrhea, abdominal pain, or unexplained hemorrhage; AND 2. An epidemiologic risk factor within the 21 days before the onset of symptoms. Epidemiologic risk includes a history of travel to a country with widespread Ebola virus transmission or contact within the preceding 21 days with a person with Ebola while the person was symptomatic.
Turn-Around-Time:	Results are reported within 6 hours after DIB approval and receipt of specimen(s).

Specimen type required:	Whole blood preserved with EDTA is preferred but whole blood preserved in sodium polyanethanol sulfonate (SPS), citrate or with clot activator is acceptable. Do not separate and remove serum or plasma from the primary collection container. Opening the tubes destroys the vacuum seal and increases the risk of leakage during transport. Urine samples may also be submitted but should not be the sole specimen tested from a patient. Urine samples should be submitted with a corresponding blood specimen. For adults, a minimum of 4 ml whole blood is preferable. For pediatric samples, a minimum of 1 ml whole blood should be collected in pediatric-sized collection tubes. **DO NOT** use glass tubes for collection. **DO NOT** use heparinized tubes.
Specimen storage/transport:	Ship specimens at 2-8°C on cold packs within 24 hours. Ship on dry ice if the specimens have been frozen at -20°C or lower. Transport specimens within the facility in compliance with 29 CFR 1910.1030. Place specimens in a durable, leak-proof secondary container for transport. Specimens in secondary containers should be hand-carried to the laboratory or packing area. **DO NOT** use a pneumatic tube system for transporting specimens. Follow instructions for Class A - Biological Substance of the U.S. Department of Transportation (U.S. DOT – 49 CFR 171-180) and International Air Transport Association (IATA) for packing and shipping. Label "Diagnostic specimen suspected to contain a Class A Biological Substance". On the outside of the box, indicate how the specimen should be stored: refrigerated, frozen, or do not refrigerate.
Specimen submission:	Submitters: Clinical laboratories and the DIB. Prior consultation and approval from the State Epidemiologist or designee is required prior to the submission of specimens. DOH will contact the CDC in order to get a PUI number, which will be used in all subsequent communications.
Criteria for rejection:	• Authorization and approval from the State Epidemiologist

and/or CDC PUI# was not secured prior to submission to the State Lab.

- 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 or special handling instruction is not followed.
- Blood collected with heparin.
- Specimen is not received at 4°C or packed in blue ice.
- Frozen specimens not shipped in dry ice.
- Specimen quantity is insufficient to perform the tests.
- Unlabeled or incomplete specimen labeling and documentation.
- Specimen label does not match the requisition.

Stability:

If the specimen cannot be transported to the SLD within 24 hours after collection, freeze at -20°C or lower. Frozen samples should be shipped in dry ice.

Requisition Form:

Each specimen submitted must have a completed SLD Form 81.3 and a Chain-of-Custody Form. Submitter is responsible for completing SLD Form 81.3 including but not limited to the following information: at least two patient identifiers, date of onset of illness, signs and symptoms, travel history, immunization history, name and address of submitter.

A completed CDC Form 50.34 is also required for the specimen that will be submitted to the CDC for confirmatory testing.

Requisition forms shall be placed in a separate bag and shall not be packed with the specimen(s).

Normal Value:

Ebola virus RNA not detected by rRT-PCR.

Disclaimers:

If fever or symptoms have been present for less than 72 hours, a repeat test may be required to rule out Ebola virus infection. If Lassa fever is a consideration (e.g. recent travel to West Africa), please refer the specimen to CDC for Lassa fever testing.

False positive can occur. A positive sample would be submitted to the CDC for additional evaluation.

Result Notification:	Laboratory results are reported to the submitters and DIB of the DOH Disease Outbreak Control Division (DOCD). Laboratory reports for the DIB will be posted to the DOCD SharePoint.
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