

## Specimen Requirements for Real-Time (rti) Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) for EUA Assay Ebola Zaire (EZ1) Virus in Clinical Samples

Methodology:	Department of Defense (DOD) Ebola Zaire (EZ1) virus rti RT-PCR Assay
Performed:	The EZ1 real-time (rti) Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) Assay is an FDA- approved Emergency Use Authorization (EUA) assay developed by the DOD to test for the presumptive presence of Ebola Zaire virus in whole blood, plasma, Trizol-inactivated whole blood, or Trizol-inactivated plasma specimens.
Criteria for testing:	<p><b><u>Mandatory consultation required.</u></b> Consultation with and authorization from the Department of Health (DOH), State Epidemiologist is required <b><u>PRIOR</u></b> to collecting and submitting specimens to the State Laboratories Division for testing. The DOH will coordinate with Centers for Disease Control and Prevention (CDC).</p> <p>This test should only be performed for individuals who have both consistent signs or symptoms and risk factors such as:</p> <ol style="list-style-type: none"><li>1. Elevated body temperature or subjective fever or symptoms, including severe headache, fatigue, muscle pain, vomiting, diarrhea, abdominal pain, or unexplained hemorrhage; AND</li><li>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.</li></ol>
Turn-Around-Time:	Results are reported within 6 hours after approval and receipt of specimen(s).
Specimen type required:	Appropriate specimens are whole blood, plasma, Trizol-inactivated blood, or Trizol-inactivated plasma, collected in lavender –top EDTA tubes is preferred but whole blood preserved in sodium polyanethanol sulfonate (SPS), citrate or with clot activator is acceptable.

Two 4 ml. tubes will be collected and submitted to the State Labs for testing. **DO NOT** use a pneumatic tube system or glass tubes for collection, to reduce breakage or leaks.

Specimen storage / transport: Ship specimens at 2-8°C or frozen on cold packs within 24 hours. Ship on dry ice if the specimens have been frozen at -20°C or lower.

Refrigerate serum or plasma at 4°C or maintain on ice for no longer than 24 hours. If storage/transport will exceed 24 hours, freeze serum or plasma at -20°C or lower.

Ship in compliance with 29 CFR 1910.1030. Place specimens in a durable, leak-proof secondary container for transport. Specimens collected for EVD testing should be packed and shipped without attempting to open collection tubes or specimens.

Pack specimens for shipment in a basic triple-packaging system consisting of a primary container (a sealable specimen bag) wrapped in absorbent material and a secondary container (water-tight, leak-proof), and an outer shipping package.

Follow instructions for Class A - Biological Substance of the U.S. Department of Transportation (U.S. DOT) and International Air Transport Association (IATA) for packing and shipping. Label "Diagnostic specimen suspected to contain a Class A Biological Substance" DO NOT label "ebola".

Specimen submission: Submitters: Clinical laboratories and the Disease Investigation Branch (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 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 specimens;
- Incomplete specimen labeling/documentation.

**Stability:** If the specimen cannot be transported to the State Laboratories Division within 24 hours after collection and separation, it should be frozen at -20°C or lower. Frozen samples should be shipped in dry ice.

**Requisition Form:** Each specimen submitted must have a completed 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: patient identifier, 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:** Negative for Ebola Zaire virus 1 (EZ1) by real-time Reverse-Transcriptase 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 Disease Investigation Branch (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 Program (LPRP)  
 State Laboratories Division  
 Department of Health  
 2725 Waimano Home Road  
 Pearl City, Hawaii 96782

Contact:

Rebecca Sciulli M.Sc., MT at 808-453-5993 or  
808-368-3373 or 554-9992.

Approved By:

*A. Christian Whelen*

\_\_\_\_\_  
A. Christian Whelen, Ph.D.  
Administrator, State Laboratories Division

*11-6-2014*

\_\_\_\_\_  
Date