



Department of Health  
**Research Institute for Tropical Medicine**  
National Reference Laboratories for Emerging Infectious Diseases  
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## **INTERIM GUIDELINES FOR COLLECTION, HANDLING, PROCESSING AND PACKAGING OF SPECIMENS FROM PERSONS UNDER INVESTIGATION (PUI) AND EVD SUSPECTED CASES**

Version 4 | 26 October 2014

### **BACKGROUND**

Ebolavirus disease (EVD) is a viral hemorrhagic fever and one of the most virulent viral diseases known to humankind. The current EVD outbreaks in affected countries in West Africa have a case fatality rate of 50%-60%. The virus is transmitted to people from wild animals and spreads in the human population through person-to-person transmission. Person-to-person transmission by means of direct contact with infected persons or their body fluids/secretions is considered the principal mode of transmission. The patient becomes contagious once they begin to show symptoms. The incubation period ranges from 2 days-21 days.

Processing samples from patients with suspected or confirmed EVD using routine biosafety precautions in diagnostic labs poses no greater risk than samples containing hepatitis B, hepatitis C, HIV and other blood-borne viruses.\*\* The significant risk for laboratory-acquired infection, when working with samples that may contain Ebola virus, is **percutaneous inoculation injury (i.e., "needlestick injury")**. While there is currently no evidence of any aerosol transmission risk from Ebola infected patients, exposure of mucous membranes to splashes of infectious material, and inhalation of infectious aerosols are still considered potential modes of acquiring EVD.

It is important that hospital staff responsible for collecting, processing and routine testing of specimens are aware of and trained in appropriate biosafety and infection control guidelines in order to minimize the risk of infection with EVD.

**Any facility that is not capable of handling these cases must refer to the nearest capable regional referral hospital and medical center.**

In the course of managing Patients Under Investigation (PUIs) and EVD Suspects that may be encountered in regional referral hospitals and medical centers capable of catering to such cases, physicians may request for some routine laboratory tests to guide general patient management.

At the same time, laboratory confirmation is needed to classify a patient as a confirmed case of Ebola Virus Disease (EVD) to initiate appropriate specific clinical management and epidemiological investigation. The Research Institute for Tropical Medicine (RITM), the National Reference Laboratory for Emerging and Re-emerging Infections, performs molecular detection by polymerase chain reaction (PCR) and Enzyme-linked Immunosorbent Assay (ELISA) antigen and antibody tests in the rapid detection of EVD.\*

This document has two parts:

- A. Guidelines for collection, handling and processing of specimens for routine diagnostic testing
- B. Guidelines for packaging of specimens for shipment to confirmatory testing laboratory

This document provides the most updated interim guidelines for the safe collection and processing of specimens from PUIs and Suspected EVD cases for routine diagnostic testing to guide clinical management, and packaging of specimens for shipment to confirmatory testing laboratory. It is based on the most recent evidence and best practices of hospitals with experience in the safe handling of EVD cases. The classification of patients as PUI or EVD Suspect cases is described in the “DOH Interim Guidelines No.1 EVD Surveillance and Reporting.”

**A. COLLECTION OF SPECIMENS FOR ROUTINE DIAGNOSTIC TESTING**

**1. General Guidelines**

- **Heads/laboratory managers of EVD regional referral hospital or medical center must perform a risk assessment to determine potential for sprays, splashes and aerosol generation. The facility exposure management plan shall depend on this risk assessment.**
- **Aside from ensuring the safety of its nursing and medical staff, an EVD regional referral hospital or medical center must ensure that the laboratory is equipped with the following basic requirements:**

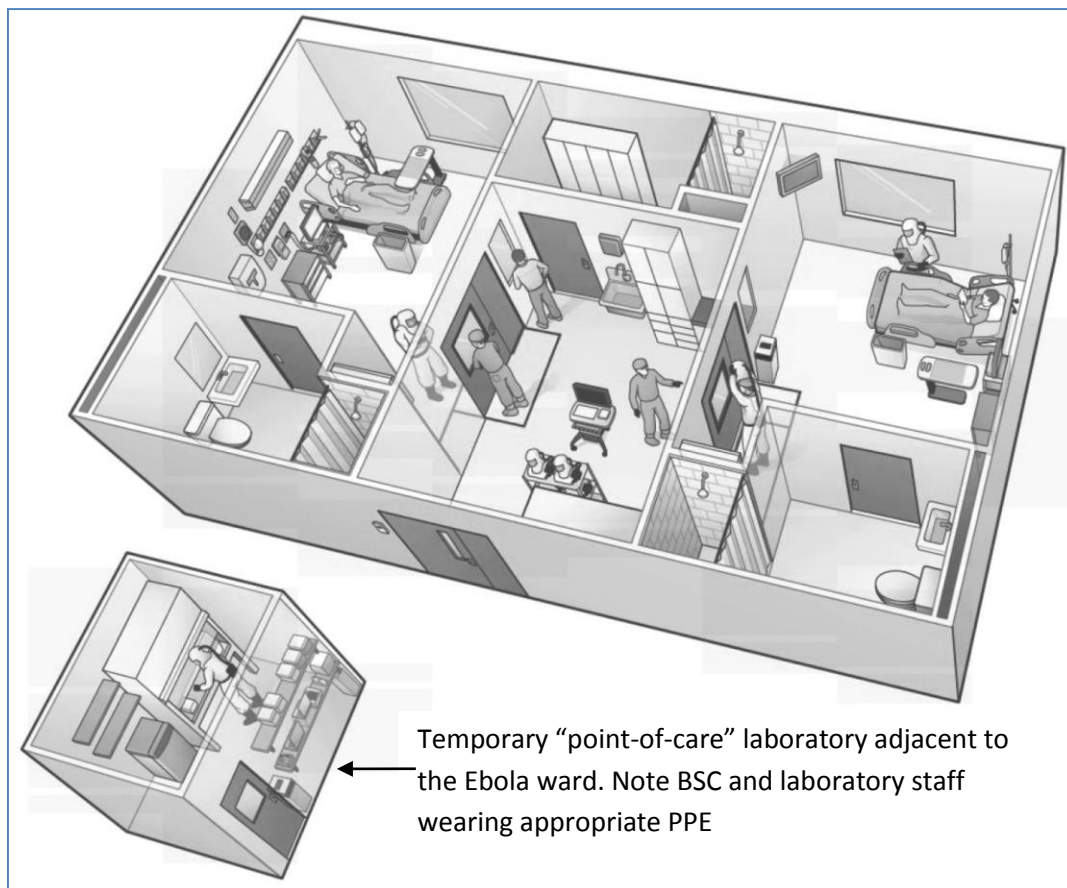
<b>BASIC REQUIREMENTS FOR ENSURING STAFF SAFETY IN SPECIMEN COLLECTION, HANDLING, PROCESSING AND TESTING OF SAMPLES FROM PUI'S AND EVD SUSPECT CASES</b>			
<b>SPECIMEN COLLECTOR</b>	<b>LABORATORY PROCESSING AND TESTING PERSONNEL</b>	<b>EQUIPMENT</b>	<b>SUPPLIES AND REAGENTS</b>
Minimum training and skill requirements: <ul style="list-style-type: none"> <li>▪ Phlebotomy</li> <li>▪ Biosafety</li> <li>▪ Donning and doffing of Personal Protective Equipment</li> </ul>	Minimum training and skill requirements: <ul style="list-style-type: none"> <li>▪ Good laboratory practices</li> <li>▪ Biosafety</li> <li>▪ Donning and doffing of Personal Protective Equipment</li> <li>▪ Use of Biosafety Cabinet</li> <li>▪ Use of Point-of-Care system (e.g., i-STAT or equivalent)</li> <li>▪ Specimen packaging and shipment</li> </ul>	<ul style="list-style-type: none"> <li>▪ Functional and certified Biosafety Cabinet Type II</li> <li>▪ Dedicated centrifuge with cover</li> <li>▪ Dedicated handheld point-of-care testing system (e.g., i-STAT or equivalent)</li> <li>▪ Appropriate Personal Protective Equipment as prescribed in these interim guidelines (see item XX)</li> <li>▪ Autoclave</li> </ul>	<ul style="list-style-type: none"> <li>▪ Supplies and reagents dedicated for routine testing of PUIs or EVD Suspect cases.</li> <li>▪ Supplies dedicated for waste management, such as sharps container, trash bags, trash bins, etc.</li> </ul>

**2. Establishment of a Temporary “Point-of-Care” Laboratory Adjacent to or Near the Isolation Rooms of PUIs/EVD Suspect Cases**

- RITM recommends that no specimens from PUIs/EVD Suspect Cases be processed and tested in the hospital’s general clinical laboratory.

INTERIM GUIDELINES FOR SPECIMEN COLLECTION, HANDLING, PROCESSING AND PACKAGING OF SPECIMENS FROM PUIs AND SUSPECTED EVD v4.0

- It is highly recommended that a temporary “point of care” laboratory adjacent to or near the isolation rooms of PUIs/EVD suspect cases be set up. This is where samples from PUIs/EVD suspect cases shall be processed and run.
- The temporary “point-of-care” laboratory shall have the following minimum required equipment:
  - Biosafety cabinet type II
  - Dedicated centrifuge with cover
  - Dedicated pipettors
  - Dedicated handheld Point-of-care testing system (e.g., i-STAT or equivalent)
  - Dedicated autoclave
- The temporary “point-of-care” laboratory shall have dedicated supplies and reagents for testing, specimen packaging and transport, spill management as well as waste management.
- Sample layout shown below:



Source: Ribner BS. (2014). Planning to Treat patients with Ebola virus infection in the US. Part of “Preparing for Ebola: What US Hospitals can learn from Emory Healthcare and Nebraska Medical Center. Clinician Outreach and Communication Activity Webinar.” October 2014.

### 3. Personal Protective Equipment

- RITM highly recommends that a room be dedicated only for donning and doffing of PPE.
- All PPE should be donned and doffed in dedicated rooms with a safety officer or trained observer assisting the staff (see “buddy system” item no. 4)
- No skin should be exposed when working in PPE.
- All staff collecting specimens from a PUIs/EVD Suspect should wear the following personal protective equipment (PPE):

Case Classification	Appropriate Attire of Collecting Staff
Person Under Investigation (PUI)	<ul style="list-style-type: none"> <li>• Attire under PPE: Scrub suit with dedicated washable footwear; No personal items (e.g., cellphone, jewelry, etc.)</li> <li>• Double layer of nitrile gloves with extended cuffs</li> <li>• Disposable full face shield</li> <li>• N95 respirator OR Powered Air Purifying Respirator (PAPR)</li> <li>• Disposable coverall</li> <li>• Disposable fluid resistant or impermeable boots or shoe covers</li> <li>• <i>Additional PPE for staff attending to patients who are vomiting or with diarrhea: Single use fluid resistant or impermeable apron.</i></li> </ul>
EVD Suspect	

- All staff processing and performing routine diagnostic tests must wear the following appropriate personal protective equipment:

Case Classification	Appropriate Attire
Person Under Investigation (PUI)	<ul style="list-style-type: none"> <li>• Attire under PPE: Scrub suit with dedicated washable footwear; No personal items (e.g., cellphone, jewelry, etc.)</li> <li>• Double layer of nitrile gloves with extended cuffs</li> <li>• Disposable Full face shield</li> <li>• Disposable coverall</li> <li>• N95 respirator OR Powered Air Purifying Respirator (PAPR)</li> <li>• Disposable fluid resistant or impermeable shoe covers</li> </ul>
Suspect EVD	

#### 4. Buddy System

- RITM highly encourages a “buddy system” in the processing and testing of specimens from PUIs/EVD Suspects.
- The safety officer or trained observer acts as the “buddy.”
- It is the role of the “buddy” to:
  - Observe for and assist the staff in case of breach in infection control procedures.
  - Make sure that infection control practices are observed at every step.
  - Remind the person of the next step.
  - Make sure no breach in protocol arises.
  - Reports any breach in protocol.

#### 5. Specimen Collection

- Specimen collection and processing should be planned ahead of time and should be timed during non-peak hours in the laboratory.
- It is important that local hospital infection control officers must be informed BEFORE the collection of specimens from PUIs and EVD suspects for routine diagnostic testing in the point-of-care laboratory.
- Ensure that all laboratory staff are trained in routine good laboratory practices, including biosafety.
- Specimens should only be obtained by trained staff experienced in specimen collection.
- Only specimens essential for diagnosis should be obtained. Specimen collection and testing should be conducted on a routine basis in a single morning session if feasible.
- As much as possible, do not use glass specimen collection devices/containers.
- All equipment for blood collection and preventing infections should be assembled BEFORE entering the patient’s room. Clearly pre-label tubes prior to the collection of patient specimens.
- All used needles should be discarded into leak-proof and puncture resistant sharps containers. All infectious wastes are placed into an infectious waste bag.

- For patients falling under the DOH Case Definition for EVD Suspect the following additional specimens are to be collected:
  - Minimum of 2 ml whole blood in **plastic** violet top tube  
AND
  - Minimum of 2 ml whole blood in **plastic** red top tube OR yellow top tube (tube with serum separator)\*

**\*NOTE: Red/Yellow top tube (tube with serum separator) requires prior centrifugation to separate the serum from the clot. Use a dedicated centrifuge with cover for this purpose. It is recommended to do all centrifugation **INSIDE** the biosafety cabinet; however, the laboratory staff must ensure that centrifugation does not interfere with the airflow characteristics within the BSC. It is important to secure the tube cover with parafilm during the centrifugation process to prevent accidental spillage. Do not uncover the tube once centrifugation is done.**

## 6. Needlestick Injury Precaution

**NOTE: Needlestick injuries can lead to serious or fatal infections. All healthcare workers who use or may be exposed to needles should take necessary steps to protect themselves.**

- **Plan for safe handling and disposal of needles prior to using them.**
- **When disposing of syringes used in blood extraction, dispose of the whole syringe with the needle intact.**
- **Do not recap needles.**
- **Do not bend the needle prior to disposal.**
- **Promptly dispose of used needles in appropriate disposal containers.**
- **If you get a needlestick injury, stop work**
- **Report all needlestick and sharps-related injuries to the hospital infection control to receive appropriate follow up and post-exposure prophylaxis.\* \*\*There is currently no post-exposure prophylaxis for EVD.**

## 7. Referral and Delivery of Specimens to the “Point of Care” Laboratory

In the referral and delivery of specimens from point of collection to the temporary “point-of-care” laboratory:

- Clinical laboratory staff must be informed PRIOR to receiving specimens from PUIs/EVD Suspects.
- All specimens shall be clearly labelled that they are taken from PUIs/EVD Suspects. This should also be clearly stated on the request form accompanying each specimen.
- The specimen container shall be surface decontaminated (see no. 11 for appropriate decontaminating agent).
- Specimens should be hand-delivered to the “point-of-care” laboratory in a durable, leak-proof/sealed transport container. The staff delivering the specimen should wear gloves.
- Where applicable, pneumatic tube systems should NOT be used to deliver specimens from PUIs/EVD Suspects to the laboratory.

## 8. Testing and Laboratory Platform Recommended

- Only point-of-care testing platform (e.g. i-STAT or equivalent) is recommended for hematology, chemistry, analytes and coagulation parameters to guide patient management.
- Only point-of-care testing platform for urinalysis is recommended.
- Specimens from PUIs/EVD Suspects are not recommended to be run in the routine analyzers of the general clinical laboratory.
- Slide preparations and blood cultures are not recommended. **If malarial smear is done, the blood film must be prepared and examined by a trained malaria microscopist who is also trained in donning and doffing of PPE and biosafety.**

- **All POC testing should be performed inside a functional, certified Biological Safety Cabinet (BSC) Type II.**

## 9. Aerosol Generating Procedures

- Minimize activities that may generate aerosols whenever possible (e.g. mixing samples by pipetting, centrifugation, aspiration, slide preparation).
- Centrifugation, for separating serum, may be carried out provided that the centrifuge is covered and the tube is sealed and decontaminated prior to loading in the centrifuge.

## 10. General Guidelines for Management of Spills

- The WHO recommendations for cleaning up spills of blood or body fluids suggest flooding the area with a 1:10 dilutions of 5.25% household bleach for 10 minutes for surfaces that can tolerate stronger bleach solutions (e.g., cement, metal). For surfaces that may corrode or discolour, they recommend careful cleaning to remove visible stains followed by contact with a 1:100 dilution of 5.25% household bleach for 15 minutes.
- The area should be evacuated and secured. Let possible aerosols settle for a minimum of 30 minutes. Accidental spills of potentially contaminated material should be covered with absorbent paper towels, liberally covered with disinfectant starting from the edge of the spill moving inward, and then left to soak for 15 minutes before being wiped up. Following the removal of the initial material, the disinfection process should be repeated.
- Individuals attending to this task should be trained and wear appropriate protective attire. As per standard laboratory spill response procedures, PAPRs or other approved respirators (e.g., N95, N100) should be worn for those involved in the clean-up activity. Disposable gloves, impermeable gowns and protective eye wear are to be removed immediately after completion of the process, placed in an autoclave bag, and sterilized prior to disposal.

## 11. Guidelines for Disinfection and Decontamination

Decontamination protocols that inactivate enveloped viruses (such as influenza or hepatitis C) will also inactivate Ebola virus. All potentially contaminated liquid and solid materials should be appropriately sterilized before disposal.

### Susceptibility to Disinfectants

- Ebolavirus is susceptible to 3% acetic acid, 1% glutaraldehyde, alcohol-based products, and dilutions (1:10-1:100 for  $\geq 10$  minutes) of 5.25% household bleach (sodium hypochlorite), and calcium hypochlorite (bleach powder).

### Susceptibility to Physical Inactivation Procedures

- Ebola are moderately thermolabile and can be inactivated by heating for 30 minutes to 60 minutes at 60°C, boiling for 5 minutes, or gamma irradiation (1.2 x10<sup>6</sup> rads to 1.27 x10<sup>6</sup> rads) combined with 1% glutaraldehyde. Ebolavirus has also been determined to be moderately sensitive to UVC radiation.

### Decontamination and Disposal of Patient Samples

- Specimens should be inactivated by autoclave on site prior to disposal.
- Patient specimens that are not for immediate disposal should be packed in clearly labelled, rigid containers, which should be surface decontaminated and retained within the laboratory awaiting safe disposal.

- Long term storage of specimens from confirmed EVD cases is not recommended.

#### **Disinfection of Work Surfaces**

- Disinfection should be done promptly after completing work on the surface.
- Work surfaces should be treated with a 1:100 dilution of 5.25% household bleach with contact time of at least 10 minutes.

#### **Patient Specimens for Confirmatory Testing at RITM**

- Specimen containers (primary AND secondary) to be transported for further/confirmatory testing to be surface decontaminated using 1:100 dilution of 5.25% household bleach prior to packaging.

### **12. Post-testing Procedures**

- Remove PPE in a dedicated PPE doffing room with a safety officer or trained observer assisting. PPE should be removed in a manner that minimizes contamination of the skin and hair.
- It is recommended to avoid any contact between soiled items (*e.g.*, gloves, gowns, respirators) and any area of the face.
- Contaminated clothing and PPE are to be appropriately sterilized.
- Perform appropriate hand hygiene immediately after the removal of PPE.
- Staff may leave PPE removal/doffing room in scrubs and dedicated decontaminated footwear.
- Showers are recommended at each shift's end for staff performing high risk patient care.

### **13. Management of Laboratory Waste**

- As a general rule, all wastes generated during laboratory testing should be placed in leak-proof containers and disinfected by autoclave BEFORE being taken out of the laboratory.
- To minimize contamination of the exterior of the waste bag, place this bag in a rigid waste container designed for this use.

### **14. Other Considerations**

#### **• Post-mortem examinations**

In patients who die of Ebola virus infection, virus can be detected throughout the body. Ebola virus can be transmitted in postmortem settings by laceration and puncture with contaminated instruments used during autopsy, through direct handling of human remains without appropriate personal protective equipment, and through splashes of blood or other body fluids (*e.g.* urine, saliva, feces) to unprotected mucosa (*e.g.*, eyes, nose, or mouth) which occur during postmortem examinations. Autopsies on patients who die of Ebola should be **avoided**.

- If there is no pre-mortem laboratory confirmation of EVD, **a trained staff wearing the prescribed personal protective equipment**, shall perform oral swab from the deceased patient, place this in Virus Transport Medium (VTM) and submit the sample per the guidelines in the next section.



## **GUIDELINES FOR SPECIMEN COLLECTION AND PACKAGING FOR SHIPMENT TO CONFIRMATORY TESTING OF EVD**

### **1. Timing of Collection of Specimen for Ebola Testing**

Ebola virus is detected in blood only after onset of symptoms, most notably fever. It may take up to 3 days post-onset of symptoms for the virus to reach detectable levels. Virus is generally detectable by real-time RT-PCR from 3-10 days post-onset of symptoms, but has been detected for several months in certain secretions. IgM antibodies can appear as early as 2 days post onset of symptoms and disappear between 30 and 168 days after infection. IgG-specific antibodies develop between day 6 and 18 after onset and persist for many years.

Specimens ideally should be taken when a symptomatic patient reports to a healthcare facility and is suspected of having an EVD exposure; however, if the onset of symptoms is <3 days, a subsequent specimen will be required to completely rule-out EVD.

### **2. Specimen Collection**

Refer to Specimen Collection Guidelines and precautions under Letter A.

### **3. Specimen Packaging**

**All packaging of specimens to be shipped to RITM for confirmatory testing of EVD should be done inside a Biosafety Cabinet Type II with the staff wearing the appropriate PPE.**

#### **Requirement for Prior Surface Disinfection of Specimen Containers**

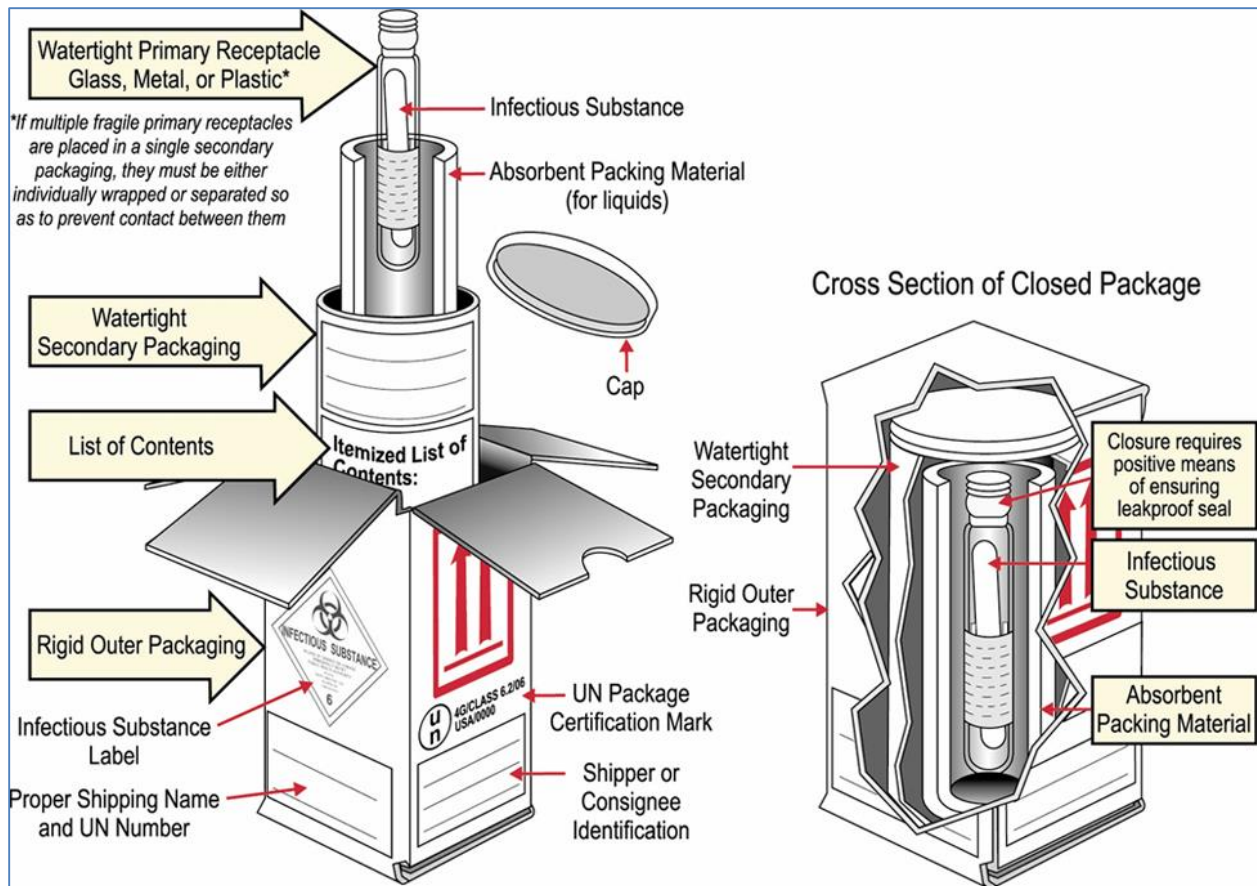
The sending laboratory is responsible for the proper surface disinfection of specimen containers, packaging of the specimens and in assuring that the specimen reaches RITM in good condition.

Specimens collected for EVD testing should be packaged and shipped without attempting to open collection tubes or aliquot specimens.

#### **Triple Packaging System**

Specimens for shipment should be packaged following the **basic triple packaging system**:

- Primary sample container (e.g., violet top and red top/yellow top tube) wrapped with absorbent material (e.g., cotton) and placed in separate resealable plastic bags
- Secondary receptacle (watertight, leak-proof container); and
- An outer shipment box



**Source:** CDC Interim Guidance for Specimen Collection, Transport, Testing, and Submission for Patients with Suspected Infection with Ebola Virus Disease. Available at: <http://www.cdc.gov/vhf/ebola/pdf/ebola-lab-guidance.pdf>. Accessed September 29, 2014.

### **Specimen Packaging Material**

RITM recommends use of IATA-approved specimen triple packaging materials suitable for Category A Infectious Substances for the shipment.

If the ideal packaging material is not readily available, the sending laboratory may use an alternative method provided that the specimen is triple packaged as described above and the outermost container bears the appropriate signages:

- Biohazard sign
- Infectious Substance Label (Category A for EVD)
- Shipper Identification
- Receiver or consignee identification
- Orientation
- Indicate **“3 packaging system inside.”**

4. **Coordination**

Under the Philippine Integrated Disease Surveillance and Response System, all referrals for shipment should be coursed through the appropriate Regional Epidemiology Surveillance Unit (RESU).

5. **Accompanying Forms**

The filled up EVD CIF and RITM Official Laboratory Request Form for Special Diagnostic Tests shall be placed in a separate zip-locked plastic bag which is sealed and secured on top of the outer shipment box with tape.

The shipment shall be addressed to:

**DR. FE ESPERANZA J. ESPINO**  
**Chief, Laboratory Research Division**  
**Research Institute for Tropical Medicine**  
**Fil-Invest Corporate City, Alabang, Muntinlupa City**

Failure to comply with the said procedures shall result in non-testing of specimens.

**NOTE: For safety considerations of the RITM staff handling the shipments, PLEASE DO NOT mix specimens for Ebola testing with other specimens to be received by other laboratories. Address specimens from routine surveillance to the laboratories concerned.**

6. **Specimen Transport Conditions**

Specimens should be transported in reverse cold chain, maintaining temperature between 5-8 °C.

Place at least six (6) frozen ice packs inside the shipment box to maintain prescribed temperature. Put the frozen ice packs in first, at the bottom and at the sides of the carrier box; then place the secondary container (containing the primary sample tubes) at the middle so that they are surrounded by the ice packs. Cover the carrier box.

7. **Confirmatory Testing at RITM**

Currently, RITM performs Antigen detection ELISA and PCR for detection of the virus during the Acute Phase of the disease and IgG ELISA for detection of antibodies during the convalescent phase of the disease.

8. **Turnaround Time and Cut-off for Specimen Receipt**

Turnaround time from receipt of specimen at RITM to result of confirmatory testing is 2 days (48 hours). Daily cut-off time for receiving of specimens for testing for the day is 10 AM.

9. **Laboratory Tests**

- For early detection of Ebola virus in suspect cases, detection of viral RNA or antigen are the recommended tests.

- If the test is positive for the presence of Ebola virus using either test, the case is laboratory-confirmed.
- Two (2) negative PCR test results, at least 48 hours apart, are required for a clinically asymptomatic patient to be discharged from the hospital.

**10. Transmittal of Results of Ebola Testing**

The Director's Office of RITM will be responsible for FAXING the results to the requesting RESU/physician while the RITM Surveillance Unit will be responsible for sending the results to the requesting RESU/physician, to NEC and HEMS-OPCEN by E-MAIL.

**As an institutional policy, RITM does not release Official Results by phone.**

**11. Storage of Clinical Samples**

Clinical samples for EVD testing shall be stored at RITM. All boxes/containers used for specimen transport will be decontaminated and disposed of by RITM as infectious waste.

**12. Contact Information**

The RITM-SU can be reached at telephone number (02)994-1887 and ritmsu@gmail.com.

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