

## **1.0 PURPOSE**

To provide instructions for the safe collection, transport and processing of specimens from patients with suspected viral hemorrhagic fever (VHF), including Ebola virus disease, Lassa fever, and others. It is imperative that specimens from suspected and confirmed patients are handled in a safe manner, with strict adherence to standard safety precautions and the protocol outlined below.

## **2.0 BACKGROUND**

VHF is associated with a number of geographically restricted viruses including Ebola virus (Ebola virus disease, formerly Ebola virus hemorrhagic fever), Lassa virus (Lassa fever), Marburg virus (Marburg hemorrhagic fever), Crimean-Congo hemorrhagic fever virus, Machupo virus (Bolivian hemorrhagic fever), and Guanarito virus (Venezuelan hemorrhagic fever). Transmission occurs by direct contact with the blood or body fluids (urine, feces, semen, breast milk, and possibly others) of an infected person or exposure to objects that have been contaminated with infected secretions. VHF is characterized by sudden onset of fever, and may be associated with headache, vomiting, diarrhea, malaise, hemorrhage, multi-organ dysfunction, and shock. The fatality rate can vary and there are no treatments or vaccines at this time. PCR testing window varies for each virus and should be assessed individually according to CDC guidelines.

## **3.0 MATERIALS AND REAGENTS**

- 3.1 Bleach diluted to 1% in squirt or spray bottles (for use in the Clinical Laboratory only) or bleach disinfectant wipes (for use in Emergency Department and on hospital floors)
- 3.2 PDI Super Sani-Cloth wipes (for POC devices only)
- 3.3 Designated labels “Danger: suspected Ebola/VHF” available at microbiology accessioning area
- 3.4 Plastic blood culture bottles (available upon request at 650-724-8632)
- 3.5 Leak-proof containers for specimen transportation (available upon request at 650-724-8632)
- 3.6 QIAamp MinElute Virus Spin Kit (Qiagen, Cat# 5774)
- 3.7 TRIzol LS Reagent (Life technologies, Cat# 10296-010)

## **4.0 LABORATORY TESTING PERFORMED**

To minimize risk of exposure, testing should be restricted only to what is essential for diagnosis and monitoring, until VHF has been ruled out.

### **4.1 Sendout Testing**

Blood samples will be sent to CDC for VHF testing. Specimens other than blood may be requested after approval by Stanford or LPCH Infectious Diseases and upon consult with the CDC by calling the Emergency

Operations Center at 770-488-7100. CDC turn-around time is 4 to 10 days once the specimen has arrived at CDC laboratory.

## 4.2 In-house Testing

The following blood tests will be offered in each laboratory section:

- 4.2.1 **Microbiology:** Blood cultures, in plastic blood culture bottles only. No stool, respiratory, or fluid cultures will be performed until VHF has been ruled out by the CDC.
- 4.2.2 **Virology:** Routine virology testing will not be performed until VHF has been ruled out.
- 4.2.3 **Chemistry:** No blood, urine or body fluid specimens will be accepted by the core chemistry laboratory. Point of care (POC) testing will be available at the bedside, to include electrolytes (Na, K, Cl, iCa), BUN, creatinine, troponin, glucose, lactate, hemoglobin/hematocrit, and blood gases (pH, PCO<sub>2</sub>, PO<sub>2</sub>, TCO<sub>2</sub>, BE, SO<sub>2</sub>). POC testing will be performed by trained nursing staff with POC devices dedicated for use with the patient with suspected VHF, labeled “Danger: Suspected Ebola/VHF”, and kept in the isolation room.
- 4.2.4 **Point of Care Testing:** Dedicated iSTAT and glucose meters that can use all validated iSTAT cartridges types are available from the POCT laboratory section. Those can be obtained by calling POCT at 650-724-8934 (only Monday-Friday between 8 AM to 11 PM, Saturdays 8 AM – 4:30 PM and Sundays 2:30 – 11:30 PM) or the Laboratory Administrator- On -Call (AOC) available 24/7 (pager 15323, phone 415-607-0621). The person requesting the devices should specify whether they will be used at SHC or LPCH because there are separate interfaces for resulting. SHC and LPCH equipment will be packaged separately for ease of dispensing. Detailed instructions of cartridges and supplies are included in each equipment package indicating that Chemistry personnel will help to give supplies 24/7.
- 4.2.5 **Hematology:** Routine automated complete blood count/differential studies will be available. Closed mode of the automated analyzers will be used to minimize exposure. For malaria testing, antigen test (BinaxNOW) and thin smear (only if BinaxNOW is negative; no thick preps) will be performed. No body fluid specimens will be accepted by the hematology laboratory.
- 4.2.6 **Coagulation:** No blood specimens will be accepted by the coagulation laboratory. Point of care (POC) testing will be available at the bedside, to include INR/PT and Activated Clotting Time (ACT) measurements, and be performed at the bedside by trained nursing staff with POC devices dedicated to use with the patient with suspected VHF, labeled “Danger: Suspected Ebola/VHF”, and kept in the isolation room.

- 4.2.7 **Transfusion service:** No blood specimens will be accepted by the transfusion service. Accordingly, therapeutic blood products issued for the duration of hospitalization will consist of:
  - 4.2.7.1 RBC: universal donor (O pos), emergency-released, uncross-matched
  - 4.2.7.2 For children and women of child-bearing age (less than 50 years of age), RBC issued will be O neg, inventory-permitting.
  - 4.2.7.3 Plasma or Cryoprecipitate: universal donor blood group AB or blood group A, depending on inventory availability
  - 4.2.7.4 Apheresis Platelets: any blood group, depending on inventory availability
- 4.2.8 **Molecular Diagnostics:** No blood specimens will be accepted until VHF has been ruled out.
- 4.2.9 **Surgical Pathology:** Biopsies will not be accepted until the patient has been ruled out for VHF.

## 5.0 COMMUNICATION

- 5.1 Notifications from Clinical Staff to Laboratory  
Clinical staff (Emergency Department or others) should consult with infectious diseases first. After consultation with ID, the ID fellow should notify the clinical pathology (CP) resident on-call (pager# 12005 carried 24/7) that there is a patient with suspected VHF. The CP resident needs to find out if the blood is being drawn by nursing or by phlebotomy. If phlebotomy draw is required, a phlebotomist will be dispatched to perform the blood specimen collection as outlined in the blood specimen collection section below. CP resident will emphasize that samples have to be hand delivered to Micro/Viro accessioning lab.
- 5.2 Internal notifications in Laboratory
  - 5.2.1 CP resident will check with ID fellow to confirm that CDC has been consulted and that the patient meets CDC criteria for VHF testing (<http://www.cdc.gov/vhf/ebola/hcp/case-definition.html>).
  - 5.2.2 CP resident will find out if the blood is being drawn by nursing or by phlebotomy.
  - 5.2.3 If phlebotomy draw is requested, CP resident will notify phlebotomy supervisor or lead on duty by calling Dispatch line at 650-497-8619 and letting them know that blood from “suspected Ebola/VHF patient” needs to be collected. A phlebotomist should be dispatched by the supervisor or lead on duty to perform the blood specimen collection as outlined in blood specimen collection

section below. CP resident will emphasize that samples have to be hand delivered to Micro/Viro accessioning lab.

- 5.2.4 CP resident will notify the Laboratory Administrator on call (pager 15323) that specimens are expected from a patient with suspected VHF. The CP resident will also notify the medical directors on call for Microbiology/Virology/Chemistry/Hematology/Coagulation/Transfusion (by pager or email) to alert them in case specimens are received in their section.
- 5.2.5 The Laboratory Administrator on call will notify section supervisors that specimens are expected from a patient with suspected VHF.
- 5.2.6 CP resident on Microbiology/Virology is responsible for following up on CDC results.

## **6.0 BLOOD SPECIMENT COLLECTION BY PHLEBOTOMIST OR NURSE**

- 6.1 Personal protection equipment (PPE) for specimen collection: full face shield or goggles, mask to cover all of nose and mouth, gloves, fluid resistant or impermeable gowns. The gowns used in clinical lab are fluid resistant.
- 6.2 Use of glass containers for specimen collection should be avoided if possible.  
Note: Plastic blood culture bottles should be requested from microbiology accession lab (650-724-8632).
- 6.3 Avoid external contamination of the specimen tube or container.
- 6.4 Spray and wipe outside surface of each specimen container with 1% bleach
- 6.5 Write “Danger: Ebola/VHF” on both specimen and requisition.
- 6.6 Each specimen should be placed into a separate sealable plastic biohazard bag. Ensure that the bag is properly sealed.
- 6.7 Laboratory requisition form for each sample should be placed in a separate pocket of the biohazard bag, not inside the sealed compartment with the sample.
- 6.8 Spray and wipe the outside of the biohazard bag with 1% bleach before leaving the patient’s room.
- 6.9 Place collected specimen into a durable, leak-proof container (available upon request at 650-724-8632) for transport to the microbiology accessioning area of the laboratory. DO NOT use the pneumatic tube system.
- 6.10 Microbiology/virology accessioning personnel will place “Danger: suspected Ebola/VHF” labels on both specimen and requisition. The labels are stored in the microbiology accessioning lab.
- 6.11 Only approved tests will be performed, all other tests will be canceled and credited unless approved by a medical director.

## **7.0 BLOOD SPECIMEN TYPES TO BE COLLECTED BY PHLEBOTOMIST OR NURSE**

- 7.1 For VHF testing:
  - 7.1.1 Consult with the CDC (<http://www.cdc.gov/laboratory/specimen-submission/list.html#L>) or Emergency Operations Center at 770-488-7100) regarding specific instructions based on the suspected VHF pathogen and patient risk assessment.
- 7.2 For Ebola virus testing:
  - 7.2.1 At least 4mL whole blood in plastic EDTA tubes (lavender top).
  - 7.2.2 Do not obtain any other specimen type.
- 7.3 For routine blood testing:
  - 7.3.1 Only those tests preapproved in the section on “Laboratory Tests Performed”/“In-house Testing” should be collected, if ordered.
  - 7.3.2 Follow established protocols and the additional guidelines in section “Blood specimen collection by phlebotomist or nurse” for collection.

## **8.0 ACCESSIONING AND SPECIMENT TRANSPORT**

- 8.1 DO NOT use the pneumatic tube system.
- 8.2 All specimens from patients with suspected VHF are to be delivered to the microbiology/virology accessioning lab by hand in sealed biohazard bags placed inside a durable leak proof container to the microbiology accessioning area of the laboratory.
- 8.3 Microbiology accessioning personnel will place “Danger: Suspected Ebola/VHF” labels on both specimen and requisition. The labels (shown below) are stored in the microbiology accessioning area (650-724-8632).

**Danger:**  
**Suspected Ebola/VHF**

- 8.4 All specimens will be recorded in a password protected by microbiology/virology accessioning supervisor (see section “Pathogen Accountability” below) and stored in the original biohazard bags, in a locked refrigerator.
- 8.5 Specimens should be hand delivered to hematology laboratory personnel and transported by the laboratory courier packaged as above to the Hillview clinical microbiology laboratory for testing.
- 8.6 Specimens will be sent for testing for VHF viruses by the Sendout Department.
  - 8.6.1 The Sendout Department should consult with Santa Clara County Public Health Officer (SCC PHO) (408-885-4214) or, if the PHO is not available, with Santa Clara County Public Health Laboratory

(PHL) (408-885-4272) regarding appropriate specimen and transport instructions. If neither the SCC PHO nor PHL is available, the CDC Emergency Operations Center (770-488-7100) can be contacted.

- 8.6.2 For specific instructions regarding packaging and shipping blood specimens for Ebola virus identification, please see Appendix I.

## **9.0 SPECIEMEN HANDLING AND PROCESSING**

### **9.1 General Safety Principles**

- 9.1.1 Opening of specimen-containing tubes should be avoided as much as possible.

If opening of specimen tubes is required, all work with patient samples should be performed in a certified class II biosafety cabinet (BSC). Always disinfect gloves with bleach before coming out of the class II BSC. Manufacturer-installed safety features for instruments should also be used.

- 9.1.2 All disposable waste must be placed in 1% bleach i.e. plastic loops, pipettes - the waste container must be left in the hood overnight

Note: If sharps are used i.e. scalpels, place in 1% bleach before discarding in sharps container and then follow routine laboratory protocol for disposal.

- 9.1.3 Environmental surfaces or inanimate objects contaminated with blood, other body fluids, secretions, or excretions should be cleaned and disinfected using bleach, either 1% solution in a spray/squirt bottle or in the form of hospital-approved bleach wipes.

- 9.1.4 10% bleach may be used for grossly soiled surfaces. The bleach solution should remain in contact with the contaminated area for a minimum of 20 minutes.

- 9.1.5 Aerosol-generating procedures such as centrifugation or aliquoting should be limited.

- 9.1.6 PPE to be worn while handling specimens:

- 9.1.6.1 Full face shield or goggles, masks to cover all of nose and mouth.

- 9.1.6.2 Masks should only be removed after gloves and gowns are removed, and hands are washed.

- 9.1.6.3 Double gloves; outside gloves are removed without skin exposure, then inside gloves removed.

- 9.1.6.4 Fluid resistant or impermeable gowns (which include gowns used at Stanford hospital laboratories).

Note: Non-disposable gowns can be re-used if they are not wet or contaminated and are stored in a designated

area marked “VHF/Ebola gowns”. If a patient tests positive for VHF/Ebola, dispose of the gowns as regulated medical waste after laboratory testing for the patient is no longer needed, in order to reduce exposure while laundering. If a patient is ruled out for VHF/Ebola, the gowns can go through the routine laundry procedure.

9.1.6.5 See Appendix II for instructions on sequence of putting on and removing PPE.

## 9.2 Specimen Storage

- 9.2.1 After processing, the primary container for samples must be externally cleaned with 1% bleach solution and repackaged like the original sample, i.e., into a sealable plastic biohazard bag ensuring that the bag is properly sealed.
- 9.2.2 Wipe the outside of the biohazard bag with 1% bleach solution.
- 9.2.3 All specimens from suspected patients (including tests that are canceled/credited and residual specimens from Hematology or microbiology if residual specimen remains) will be stored in a designated locked refrigerator in microbiology/virology accessioning laboratory until the patient is ruled out for VHF.
- 9.2.4 Canceled specimens will be stored until the medical director of the laboratory section has given approval to discard the specimen by the designated method, see section “Specimen Disposal” below.

## 9.3 Waste Disposal

- 9.3.1 All disposable materials that have come in contact with specimens from a patient with suspected Ebola/VHF must be placed in a container with 1% bleach. The waste container should be labeled with “Danger: Suspected Ebola/VHF” and left in the class II BSC overnight, then follow routine laboratory protocol for disposal of biohazard waste.
- 9.3.2 All sharp objects (sharp pipettes, disposable plastic pipette tips, needles, broken glass, etc.) are placed in 1% bleach first before placing in a designated puncture resistant biohazard sharps container.
- 9.3.3 All waste including patient specimens and materials used for processing patient samples, must be double bagged (orange bags) and autoclaved.
- 9.3.4 Bags must only be filled to half full. Wipe the outside of the autoclave bags with 1% bleach.
- 9.3.5 Waste must be autoclaved within the timeframe according to laboratory procedures.

## 10.0 MANAGEMENT OF SPILLS

- 10.1 Cover accidental spills of potentially contaminated material with an absorbent pad saturated with 1% bleach. Allow pad to soak for 30 minutes and wipe up with absorbent material soaked in 1% bleach solution.
- 10.2 Should a large spill occur, personnel should place absorbent material on or around the spill and immediately leave the spill area but ensure that the area remains secure and no other persons go near the contaminated area.
- 10.3 Notify and consult with Occupational Health and Safety (tel# 1-STIX; pager# 1-7849).
- 10.4 Dispose the waste in a biohazard bag. With the help of an assistant, this bag should be placed inside another biohazard bag and sealed with tape for disposal.
- 10.5 If accidental spills of potentially contaminated material result in aerosol formation (e.g., major spills outside class II BSC), evacuate the laboratory and notify Occupational Health and Safety (tel# 1-STIX; pager# 1-7849) immediately.

## **11.0 MANAGEMENT OF EXPOSURE**

- 11.1 Laboratory staff accidentally exposed to potentially infected material should immediately wash the exposed part with soap and water.
- 11.2 Mucous membranes (e.g., conjunctiva) should be irrigated with copious amounts of water or eyewash solution.
- 11.3 If there is heavy contamination of clothing, the contaminated clothing must be discarded in the laboratory and the person should shower immediately.
- 11.4 The exposed person should be assessed by a physician as soon as possible for postexposure management for all appropriate pathogens (e.g., Human Immunodeficiency Virus, Hepatitis C, etc.). Contact Occupational Health and Safety during regular work hours (tel# 1-STIX; pager# 1-7849) or the Emergency Department after work hours.
- 11.5 An incident report must be completed.
- 11.6 The incident should be reported to the Santa Clara County Public Health Officer (SCC PHO) (408-885-4214) or, if the PHO is not available, the Santa Clara County Public Health Laboratory (PHL) (408-885-4272) within 24 hours. If neither the SCC PHO nor PHL is available, the CDC Emergency Operations Center (770-488-7100) can be notified.