Recommendations for Handling/Testing Specimens from Cases, or Suspected Cases, of Viral Hemorrhagic Fever for Sentinel Laboratories

(*These revisions have been made by a CLP subcommittee after collaboration with CDC personnel)

On January 30, 2015, the Centers for Disease Control and Prevention (CDC) issued updated guidance for the collection, transportation, and submission of clinical specimens for Ebola virus testing, which are accessible here: http://www.cdc.gov/vhf/ebola/healthcare-us/laboratories/specimens.html. The Committee on Laboratory Practices (CLP) of the ASM acknowledges that specimens from patients with suspected viral hemorrhagic fevers (VHF) may arrive in routine clinical laboratories without the knowledge of the laboratory, and agrees with the CDC that laboratory testing must always follow standard precautions. However, it is strongly advised that providers first notify the laboratory and the local health department prior to the submission of specimens for suspected cases of VHF.

The updated CDC guidance details minimal testing recommended for Ebola Assessment Hospitals (EAH) and Ebola Treatment Centers (ETC). This document is intended for non-EAH/ETC sentinel laboratories and outlines enhanced precautions that some institutions may choose to adopt in an abundance of caution in order to assure the safety of their testing personnel and to provide appropriate medically necessary laboratory testing to suspect VHF patients. Sentinel laboratories should not attempt to culture or test for Ebola virus. Please defer to the CDC guidance related to risk assessment/mitigation, personal protective equipment, decontamination, specimen storage and waste management (http://www.cdc.gov/vhf/ebola/healthcare-us/laboratories/index.html).

Here, specific details for laboratory test approaches are discussed, some of which are not currently addressed by the CDC. This document is presented as one possible approach for diagnostic testing of patients with suspected VHF. Guidelines for testing should be thoroughly discussed with the appropriate medical personnel prior to implementation and may include significant modifications of protocols recommended in this document. Contact your public health laboratory for any questions regarding VHF testing and submission of specimens to the CDC.

General Considerations:

A. Initial testing of patients upon presentation may be limited to CDC-required tests for confirmation of Ebola, or other VHF diagnosis. Additional testing, as medically indicated, may be determined upon consultation with Infectious Diseases and Microbiology. Any referral testing from suspect patients must be discussed with the referral laboratory prior to submitting specimens.

B. All specimens taken from the patient should be labeled as ‘SUSPECTED VHF’.

C. See Table 1 (below) for detailed descriptions of testing that may be considered after consultation.

D. Testing that requires specimen removal from a patient’s room and transport to the laboratory should be kept to a minimum (DO NOT USE A PNEUMATIC TUBE SYSTEM).

E. After appropriate risk assessment, specimen processing and/or testing may be performed in the patient’s room, nearby in a contained testing area within a biological safety cabinet (BSC), or in a BSL-2 laboratory using BSL-3 precautions. Specimen processing should be performed while wearing appropriate PPE (impermeable gown, double gloves, eye protection, N-95 mask, shoe covers). PPE should not be re-used or leave the testing area. Double-bag all PPE by placing items into bags containing bleach-soaked pads, and then place the sealed bags into a rigid plastic, impervious container for disposal. See additional guidance from the CDC.

F. Laboratories should consult their laboratory safety officer, industrial hygienist, medical waste contractor, and/or water treatment facility to determine if there are institution, state, contractor-specific requirements for disposal of wastes from patients suspected of having VHF.
<table>
<thead>
<tr>
<th>Test</th>
<th>Recommendation</th>
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<tbody>
<tr>
<td>Wipe specimen containers</td>
<td>Wash specimen containers with a laboratory bleach solution, place into a double-bag that contains absorbent pads soaked with bleach then place in a biohazard rigid transport container. All transport containers should be wiped down with a fresh 10% bleach solution prior to leaving the patient’s room. Be careful not to remove patient identifying information. Laboratory processing and/or testing of specimens may take place in the patient’s room or as described above in General Considerations: E.</td>
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<tr>
<td>Chemistry, Coagulation,</td>
<td>See Appendix 1 in CDC guidance for more details (<a href="http://www.cdc.gov/vhf/ebola/healthcare-us/laboratories/safe-specimen-management.html">http://www.cdc.gov/vhf/ebola/healthcare-us/laboratories/safe-specimen-management.html</a>).</td>
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<td>Hematology</td>
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<td>Urinalysis</td>
<td>Urinalysis (dipstick tests) may be performed in the patient’s room or in a BSC in the laboratory.</td>
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| Malaria Testing:            | Rapid Malaria antigen testing may be performed in the patient’s room by qualified laboratory personnel. It should be noted that this assay is not as sensitive as malaria smears. Thin Smears:  
  1. Collect in a lavender-top (EDTA) blood collection tube.  
  2. Wipe the outside of the tube with bleach prior to removing from the patient’s room (be careful not to remove patient identifying information). The processing steps 3 & 4 below should be done as described above in General Considerations: E.  
  3. Remove stopper of lavender (EDTA) blood tube with a gauze wipe soaked in bleach to prevent aerosol formation.  
  4. Prepare at least five thin blood films, fix in methanol for 15 minutes.  
  5. The smears can then be removed from BSC and stained with Giemsa and read as usual.  
  NOTE: Thick smears for malaria diagnosis may be done at the discretion of the laboratory director, based on the perceived clinical utility and risk assessment. For additional guidance for preparing and inactivating thick smears see CDC guidance.  |
| Blood Cultures:             | Once received in the laboratory, all specimens should be opened as described above in General Considerations: E. Wipe the outside of the bottles with bleach and inspect for any signs of breakage and positivity before loading onto the blood culture instrument or placing into an incubator for manual incubation. Importantly, the bottles should be labeled appropriately (i.e., “Possible VHF”) or segregated in the blood culture instrument/incubator. If the blood culture bottles are flagged as positive, or if they show any sign of positivity upon visual inspection, unload the bottles from the instrument or remove from the incubator, place the bottle(s) into a double-bag that contains absorbent pads soaked with bleach, place in an impervious, rigid-plastic biohazard container and process as described above in General Considerations: E.  
  1. Prepare slides for Gram stain examination and allow them to dry.  
    a) Consider using a blood culture venting and subculture device to minimize sharps-related injuries during blood culture manipulation.  
    b) Fix the blood smear in methanol for 30 minutes, followed by dry heat at 95°C for 1 hour to inactivate the specimen. Perform testing of the Gram stain QC smear in this same manner. Note there are not specific inactivation data available for filoviruses in blood culture bottles, so these times are recommended out of excess caution.  
    c) The smears can then be stained and read as usual.  
  Do not perform any direct testing (i.e., rapid molecular tests) on positive blood cultures unless it can be safely done in a BSC.  
  Inoculate plates as per protocol based on Gram stain result.  
    1. Use shrink seal (Parafilm® or other suitable plate wrap) on all sub-cultured plates, place plates in a biohazard baggie and incubate in the AFB suite (if available) in a 35°C incubator.  
    2. Examine plates for growth twice per day.  
    3. Perform all spot testing and inoculations of appropriate ID/AST systems from isolated colonies. If any growth occurs, consider subculturing the organism (as described above in General Considerations: E.) onto fresh plates and incubate overnight. Work only from the sub-cultured plates to minimize risk of contact with blood from the patient. |
Other specimens for bacterial culture:

Unless critically needed, do not perform.

Prepare (and transport) all specimens leaving the patient’s room as previously described using a freshly made 10% bleach solution. All specimens should be processed as described above in General Considerations: E.

If centrifugation is necessary, use covered carriers as for AFB processing. Sealed rotors or cups should only be opened within a BSC. If specimens show signs of breakage or leakage – do not open. Consult with the Laboratory Director.

Gram stains may be prepared as directed in the Blood culture section above.

Seal culture plates. Perform all spot testing and inoculations of appropriate ID/AST systems from isolated colonies. If any growth occurs, consider subculturing the organism (as described above in General Considerations: E.) onto fresh plates and incubate overnight. Work only from the subcultured plates to minimize risk of contact with blood from the patient.

Links and References:


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