AMERICAN SOCIETY FOR MICROBIOLOGY

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Interim Laboratory Guidelines for Handling/Testing Specimens from Cases or Suspected Cases of Hemorrhagic Fever Virus (HFV)

On August 5, 2014, the Centers for Disease Control and Prevention (CDC) issued an Interim Guidance for Specimen Collection, Transport, Testing, and Submission for Patients with Suspected Infection with Ebola Virus Disease which can be seen here http://www.cdc.gov/vhf/ebola/hcp/interim-guidance-specimen-collection-submission-patients-suspected-infection-ebola.html.
The Laboratory Practice Committee of the ASM has drafted this document which includes enhanced precautions to assist laboratory personnel to follow those guidelines currently being recommended by the CDC. This document is presented as one possible approach to testing of patients with suspected HFV. 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 State Health Laboratory for any questions regarding VHF Testing and submission of specimens to the CDC **(SEE NOTE BELOW).**

General Recommendations:

A. Initial testing of patients upon presentation will be limited to CDC required tests for confirmation of Ebola or other HFV diagnosis. Additional testing determined upon consultation with Infectious Diseases (ID) and Microbiology.
B. All specimens taken from the patient must be labeled as **'SUSPECTED HFV'**. OPTION: A chain of custody form may accompany all specimens.
C. See Table 1 below for detailed description of testing that may be available after consultation.
D. Testing that requires specimen removal from patient's room and transport of samples to laboratory should be kept to a minimum (**do not use a pneumatic tube system**).
E. All specimen manipulations must be performed in the patient's room, nearby in a contained testing area, or inside a biological safety cabinet [BSC] in an AFB suite or isolated area of the laboratory while wearing appropriate PPE (impermeable gown, double gloves, eye protection, N-95 mask, shoe covers). No PPE should be re-used or leave the testing area. Place all PPE into a double-bag which contains absorbent pads soaked with bleach, then placed in a rigid plastic, impervious container for disposal (see disposal instructions at the end of this document).

Table 1 - Testing and Laboratory Procedures:

Test	Recommendation
Specimen containers must be wiped with laboratory bleach solution, placed into a double-bag that contains absorbent pads soaked with bleach, then placed in a biohazard rigid transport container. All transport containers must be wiped down with bleach prior to leaving the patient's room. OPTION: A Chain of Custody Form may accompany all specimens. Laboratory processing of specimens should take place in the patient's room or in a class II biosafety cabinet (BSC) located in an isolated section of the laboratory or, preferably, in a negative pressure room (e.g., AFB suite). OPTION: Document receipt, referral and disposal of all specimens.	
Chemistry, Coagulation, Hematology	Testing should be limited to iSTAT or equivalent POC testing systems and performed in the patient's room (may limit to high risk or known patients).
Urinalysis	Urinalysis available as a urine dipstick should be performed in the patient's room.

Malaria Testing: Rapid Malaria antigen testing is preferred; test in patient's room.	1. Collect in a lavender top (EDTA) blood tube. 2. Only thin blood smears should be prepared (no thick smears). 3. Wipe the outside of the lavender (EDTA) blood tube with bleach prior to removing from the patient's room (be careful not to remove patient identifying information). The remaining steps should be done inside a BSC located in a negative pressure room (e.g., AFB suite) or in a remote section of the laboratory. 4. Remove stopper of lavender (EDTA) blood tube with a gauze wipe soaked in bleach to prevent aerosol formation. 5. Prepare a thin blood film, fix in methanol for 30 minutes, then place in dry heat at 95^{o}C for 1 hr. to inactivate the specimen. 6. The smears then can be carefully removed from the AFB suite/BSC. 7. Stain with Giemsa and read as usual. 8. WBC and platelet count can be estimated from the stained blood film.
Blood Cultures: Perform only if required and minimize blood draws for blood cultures. Use plastic bottles if available.	Once received in the laboratory, all specimens should be opened inside a BSC, preferably inside a negative pressure room (e.g., AFB suite). 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. 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 a biohazard rigid plastic impervious container and deliver to the AFB suite and/or BSC in isolated section of the laboratory. 1. Prepare slides for Gram stain examination and allow to dry. 2. Fix the blood smear in methanol for 30 minutes, followed by dry heat at 95^{o}C for 1 hour to inactivate the specimen. Perform testing of the gram stain QC smear in this same manner. 3. The smears can then be carefully removed from the AFB suite/BSC and processed and read as usual. **Do not perform any direct testing on positive blood cultures.** 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 the 35^{o}C CO_2 incubator. 2. Examine plates twice per day. 3. If any growth occurs, subculture the organism in the BSC onto fresh plates and incubate overnight. 4. The next day perform all spot testing and inoculations of appropriate ID/AST systems. Work only from the sub-cultured plates (not the primary inoculated 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 in the patient's room as previously described using laboratory bleach. All specimens should be opened inside a BSC, in an isolated section of the laboratory, preferably inside a negative pressure room (e.g., the AFB suite). If centrifugation is necessary, use covered carriers as for AFB processing. If specimens show signs of breakage or leakage – **do not open**. Consult with the Microbiology or Laboratory Director. Gram stains may be prepared as directed in the Blood culture section above. Seal culture plates. Subsequent secondarily sub-cultured colonies may be inoculated into the appropriate ID/AST systems.

Specimen storage:	All specimen containers should be wiped with bleach, placed into double-bags that contains absorbent pads soaked with bleach, then placed in a rigid plastic, impervious container and isolated until they can be disposed of in an appropriate manner (see specimen decontamination and disposal section below). Long-term storage of specimens is not permitted for any suspect HFV patient.
Specimen decontamination and disposal	All specimens should be autoclaved prior to disposal. If no autoclave is available on site, contact the laboratory director for procedures for discarding of specimens and other laboratory waste. OPTION: Documentation of disposal may be indicated on a Disposal Form.

NOTE: Contact information for state health laboratories can be found here: http://www.aphl.org/aphlprograms/preparedness-and-response/Pages/Emergency-Lab-Contacts.aspx.
Additional information regarding procedures for the collection, handling, and testing of specimens for EVD (Ebola) and sending specimens to the CDC for EVD testing have been issued by the CDC and are posted at the following site:
http://www.cdc.gov/vhf/ebola/hcp/interim-guidance-specimen-collection-submission-patients-suspected-infection-ebola.html
and http://www.cdc.gov/ncezid/dhcpp/vspb/specimens.html

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This Interim Guidance is advisory only and should be regarded as a guide that the user may or may not choose to adopt, modify, or reject. The acceptance or use of this Interim Guidance is completely voluntary, and is not intended to be used in place of any federal, state, or other territorial governmental standards or regulations that may apply to this topic or subject matter. Guidelines for testing should be thoroughly discussed with the appropriate medical personnel prior to implementation.