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|  | **Microbiology Testing of Specimens from Potential Ebola Patients** |
| Department of Microbiology  |
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# Introduction

As outbreaks of Ebola occur, it is anticipated that there will be patients with febrile illness who will return to the United States from outbreak areas.  However, the overwhelming majority of these patients will not have Ebola or other viral hemorrhagic fevers (VHF), but many will be sick and will require timely diagnostic testing.  It is important to recognize that the application of overly restrictive testing approaches that go beyond the interim CDC guidance could be detrimental to patient care and might result in adverse outcomes.

In order to ensure the safety of laboratory staff and other healthcare personnel collecting or handling specimens, CDC recommends that established OSHA bloodborne pathogens standards be followed. During specimen collection, CDC recommends using personal protective equipment such as full face shield or goggles, masks to cover all of the nose and mouth, gloves, and fluid-resistant or -impermeable gowns. Additional personal protective gear might be required in certain situations, such as aerosol generating procedures. During laboratory testing, CDC recommends using personal protective equipment (PPE) such as full face shield or goggles, masks to cover all of the nose and mouth, gloves, fluid-resistant or -impermeable gowns, and use of a certified class II Biosafety cabinet or Plexiglas splash guard, as well as manufacturer-installed safety features for instruments. If hospitals follow OSHA standards, and if lab personnel use appropriate personal protective equipment and adhere to engineered standards, they will not need additional safety measures. Portions of the ASM guidelines are more restrictive than the CDC guidelines. Because specimens from suspect HFV patients may arrive in the lab without the lab personnel’s knowledge, all laboratory testing must follow standard precautions, at a minimum. The additional personal protective gear and special equipment used by CDC lab workers in their BSL4 labs are because of the additional testing and experiments used to investigate the Ebola virus, including virus isolation in cell culture, small animal studies to determine pathogenicity, and production of reagents.

# Procedure

Follow the general Ebola Virus Disease Safety Guidelines for the laboratory for specimen collection, transport, and specimen processing.

Microbiology testing personnel should wear double gloves, a fluid-resistant or impermeable gown/lab coat, a full face shield or goggles, and an N95 mask to cover all of the nose and mouth AND use a certified class II biosafety cabinet in the BSL-3 lab, when performing any of the work outlined below on any specimens from patients with possible Ebola.

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| Blood Cultures:Perform only if required and minimize blood draws for blood cultures. | Wipe the outside of the bottles, including the rubber septum, with bleach and place an orange dot on top of the septum, and inspect for any signs of breakage and positivity before loading onto the blood culture instrument. 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, and place the bottle(s) into a Zip lock bag in a rigid plastic container, and transport to a biosafety cabinet in the BSL-3 lab. No work should be performed until the director and supervisor are informed of positive cultures. 1. Prepare slides for Gram stain, and allow to dry under the hood.
2. Fix the blood smear in methanol for 30 min. Perform testing of the gram stain QC smear in this same manner.
3. The smears can then be stained and read as usual.

**Do not perform any direct testing, such as disk diffusion or tube coagulase or BioFire, on positive blood cultures.** Working in the biosafety cabinet, inoculate plates as per protocol based on Gram stain result.1. Use tape on all sub-cultured plates; place plates in a biohazard bag, and incubate in the AFB room in the 35oC CO2 incubator. Use gas packs for anaerobic subcultures.
2. Examine plates for growth in a biosafety cabinet in the AFB Room.
3. Perform all spot testing and inoculations of appropriate ID/AST systems from isolated colonies in a biosafety hood in the BSL-3 lab. This work will be performed in consultation with the director and supervisor on a case by case basis.
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| Other specimens for bacterial culture: **Unless critically needed, do not perform.**  | 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 Director or supervisor. 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 in a biosafety hood in the AFB room.  |
| Specimen storage: | All specimen containers should be wiped with bleach, placed into Zip lock bags, then placed in a rigid plastic container and isolated until they can be autoclaved. Long-term storage of specimens is not permitted for any suspect Ebola patient.  |
| Specimen disposal | All specimens and cultures should be autoclaved prior to disposal.  |

# References

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

<https://www.asm.org/images/PSAB/Ebola9-10-14.pdf>

1. CDC Guidelines

<http://www.cdc.gov/vhf/ebola/hcp/interim-guidance-specimen-collection-submission-patients-suspected-infection-ebola.html>

1. Canadian Guidelines



1. Australian Guidelines

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# Document Control History

Written by:

Ann Robinson, Ph.D., DABMM, FAAM

Director, Microbiology

Providence Sacred Heart Medical Center & Children's Hospital

Spokane, WA 99220-2555