

## TRAINING UPDATE

**Lab Location:** SGAH & WAH  
**Department:** Field Ops Staff

**Date Distributed:** 1/12/2015  
**Due Date:** 2/15/2015

### DESCRIPTION OF PROCEDURE

**Name of procedure:**

**Hospital Guidelines for Handling Clinical Specimens Suspected of Ebola Virus Disease**  
**GEC.L230 / SGAH.L893 / WAH.L892 v0**

**Description:**

**Specimens suspected of Ebola virus will NOT be collected by lab Phlebotomists and will NOT be handled / processed by Specimen Processors**

**The goal of this review:**

- Read the SOP
- Specifically note the step for not referring samples to other labs
- Note that only the Testing Tech (TT) actually handles the samples
- If notified suspected Ebola samples are coming –
  - Non-lab staff are asked to leave lab areas
  - Signs are posted at all entrances

**This SOP has already been implemented**

**Document your compliance with this training update by taking the quiz in the MTS system.**

Non-Technical SOP

<b>Title</b>	<b>Hospital Guidelines for Handling Clinical Specimens Suspected of Ebola Virus Disease</b>	
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<i>Refer to the electronic signature page for approval and approval dates.</i>		
Local Issue Date:		Local Effective Date:

<b>Review:</b>		
<b>Print Name</b>	<b>Signature</b>	<b>Date</b>

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**1. PURPOSE**

This policy is designed to ensure the safe and proper handling of clinical specimens received by Quest Diagnostics laboratories at Adventist Hospitals, from patients with suspected Ebola virus Disease (EVD). General guidelines for other high virulence organisms can be found in the QDMI815 policy.

**2. SCOPE**

The policy applies to the Adventist Healthcare clinical laboratories, Shady Grove Medical Center, Washington Adventist Hospitals and the Shady Grove Adventist Emergency Center at Germantown. This guidance describes enhanced supplemental handling and/or disinfection procedures above and beyond the Standard Precautions outlined in the Quest Diagnostics Bloodborne Pathogens Exposure Control Plan and the Comprehensive Microbiology Safety Procedures documents.

**3. RESPONSIBILITY**

Responsible Party	Task
Laboratory Director, Managers and Supervisors	<ul style="list-style-type: none"> <li>Implement this policy and ensure that all lab personnel likely to encounter samples with suspected virulent organisms are trained in the special handling, decontamination and post-exposure steps. Retraining will be provided as needed to ensure competency in the process.</li> </ul>
Group Lead/Tech in Charge	<ul style="list-style-type: none"> <li>Identify and assign testing personnel who will perform the duties of Testing Tech (TT) and Assisting Tech (AT) when a sample from a suspected EBD patient is in the laboratory.</li> </ul>

#### 4. DEFINITIONS

BSL-2	Biological Safety Level 2
BSC	Biological Safety Cabinet
EPA registered disinfectant	10% bleach or Clorox® Healthcare Bleach Germicidal Cleaner, or PDI Super Sani-Cloth Germicidal Disposable Wipe
GL/TIC	Group Lead/Tech In Charge
TT	Testing Tech. This is the person who handles the suspect EBD sample throughout the testing process.
AT	Assisting Tech. This person is the observer of the TT as well as the assistant for the donning and doffing of PPE, for processing the sample and for decontaminating any surfaces outside of the BSC hood that may be come in contact with the sample. This person does not touch the sample being tested.
PUI	Person under investigation as defined by the CDC as someone with symptoms of EVD as well as a high risk or low risk exposure including travel to an EVD endemic area.

#### 5. PROCEDURE

##### General Considerations before handling these specimens:

All employees are required to adhere to the Quest Diagnostics “Standard Precautions” for handling any sample in the *Bloodborne Pathogens Exposure Control Plan, the Shady Grove Adventist Bloodborne Pathogen Exposure Control Plan (Policy #101-02-036), the 2014 WAH Infection Prevention Plan (Policy #WAH 7002), and the Adventist Healthcare Ebola Management Plan.*

Quest Diagnostics laboratories at Adventist Healthcare facilities will rely on guidance from the Medical staffs of each hospital, the Infection Control Coordinators at each facility, the CDC and local public health departments, when working with any new emerging pathogen. This policy will be updated when guidelines or information becomes available.

##### A. Ebola Virus

###### Background

Ebola virus is the cause of a viral hemorrhagic fever disease called Ebola Hemorrhagic Fever (Ebola HF) or Ebola Viral Disease (EVD) is caused by infection with a virus of the family *Filoviridae*, genus *Ebolavirus*. There are 5 known species of ebolavirus; Zaire ebolavirus, Sudan ebolavirus, Tai Forest ebolavirus, Bundibugyo ebolavirus, and Reston ebolavirus.

All but Reston ebolavirus are known to cause disease in humans. Symptoms include: fever, headache, joint and muscle aches, weakness, diarrhea, vomiting, stomach pain,

lack of appetite, and abnormal bleeding. Symptoms may appear anywhere from 2 to 21 days after exposure to Ebola virus though 8-10 days is most common.

Ebola is transmitted through direct contact with the blood or bodily fluids from an infected symptomatic person or through exposure to objects (such as needles) or surfaces that have been contaminated with infected secretions. It may be transmitted via aerosolized droplets of an infected patient's body fluids so caution is necessary when performing procedures that have the potential to aerosolize droplets. It is not transmitted through the air, or by food or water.

## B. Specimens and Tests Offered:

1. Testing in this laboratory will be limited to only those tests deemed essential for ruling out the diagnosis of EVD. After consultation with the medical staffs and infection control physicians, the following tests can and will be performed:
  - CBC
  - BMP
  - PT
  - Malaria Screen
2. Testing that will **not** be performed on suspect EVD patients because of equipment limitations in the laboratory and for reasons of staff safety include:
  - Blood Cultures
  - Differential counts
  - Blood Bank T&S [Note: Emergency Release Blood, plasma, and platelets will be provided as needed].
3. **Referral of Specimens to another Laboratory**
  - Specimens from suspected EVD patients may not be sent to another laboratory for testing without approval of the receiving lab.
  - **CDC laboratory has approved testing for EVD and Specific testing for Ebola virus will only be authorized by the CDC.** If Ebola testing is approved by CDC, someone from MDHMD or CDC will arrange to pick-up the sample.

## C. Pre-Testing General Considerations:

1. Per the hospital guidelines, the Laboratory will be notified immediately of a suspect Ebola case by the nursing staff, prior to submission of any samples for lab testing.
2. **Laboratory or phlebotomy staff WILL NOT enter a room containing a patient suspected of Ebola.** Laboratory phlebotomists **WILL NOT** collect blood from suspected or confirmed Ebola patients.
3. **Specimen Transport from nursing units to laboratory:** Samples will be drawn by hospital staff in the isolation room. These will be labeled with the Cerner label and then the outside of the tubes will be decontaminated with an approved

disinfectant. The samples will then be wrapped in a protective wrap, placed within a solid, impermeable, leak-proof, screw-cap, plastic or metal carrier container which contains absorbent material and then the exterior surface of the container will be labeled with the patients name and then disinfected with EPA approved disinfectant. All containers will be hand carried to the laboratory. **DO NOT USE THE PNEUMATIC TUBE SYSTEM.**

4. All laboratory testing will be done inside a BSC by the TT who is appropriately gowned and gloved. Uncapping any blood or specimen tube/container must take place within a Class II Biological Safety Cabinet (BSC), using BSL-2 safety practices (Addendum A) to include wearing the necessary PPE:
  - Hood covering the entire head and neck
  - Hospital-issue scrubs
  - Impermeable gown, with back closure
  - Double gloves: one pair of extended wrist gloves, surgical or others, worn next to the skin and covered with a second pair of standard laboratory gloves, which will be disinfected and changed throughout the testing process
  - Face Shield
  - N95 mask that is fit tested
  - Shoe covers

Testing equipment will remain inside the BSC hood until the patient is established as not having EVD at which point equipment is properly decontaminated and readied for new patients. If the patient is established to have EVD, the equipment will be properly decontaminated after the patient is discharged. Refer to the Ebola Processing Checklist (see Related Documents)

5. The TT will not touch any surfaces or equipment outside of the BSC including laboratory keyboards. Any computer generated processes, such as receiving samples and reporting laboratory results will be performed by the assisting tech.

#### **D. Notification of Sample Arrival**

1. When a patient with suspected Ebola presents at the hospital, the laboratory will be notified by telephone. The Group Lead/Tech-in-charge (GL/TIC), will contact the Laboratory Director immediately. The GL/TIC will designate two persons including him/herself as the TT and AT.
2. The TT and AT will go to the donning area in the laboratory and assist each other in proper donning of PPE equipment. Refer to the donning PPE procedure in Addendum C.
3. The trained observer will pay specific attention to each step of the process watching for any areas of open skin surface that is not covered by the PPE. The TT and AT will inspect each other before leaving the donning area to begin receiving and testing the sample.

4. Upon notification of a suspect EVD sample coming to the laboratory, any non-laboratory staff present within the laboratory will be asked to leave immediately.
5. The assisting tech will ensure that all laboratory entrances will have posted signs that warn staff of high risk sample testing and instruct lab staff to limit access to the laboratory testing area. Specimen handling will be limited to the TT only.

#### **E. Receiving the Sample**

1. Upon arrival at the laboratory, the TT will verify that the exterior of the container has been disinfected and accept the sample from the delivery nurse.
2. The sample transport container will be placed inside the BSC. The TT will wipe the exterior of the container with a disinfectant and discard the wipe into the designated biohazard waste container within the hood.
3. The TT will open the container and check for leaks, breakage or any evidence of loss of container integrity.
  - a. If there is evidence of blood contamination outside the specimen tube, stop the process and ask the AT to inform the floor and ask for a new sample. Discard the entire container into the biohazard waste container.
  - b. If the sample is intact, identify the specimens within, remove the specimen tubes and wipe the tubes/container with another EPA registered disinfectant wipe. Discard the wipe into the designated biohazard waste container inside the BSC.
4. The TT will call out the accession numbers and the two patient identifiers to the AT, who will repeat back each identifying detail, and will receive the specimens into the LIS.
5. The AT will print the LIS sample labels and deliver them to the tester by dropping them into the hands of the TT without touching the TT or inside of the BSC.
  - a. If the AT touches any surface potentially contaminated, he/she must proceed to the doffing area and remove gloves or any clothing that came into contact with the sample, the inside of the BSC or the TT.
  - b. The AT will remain 3 feet from the TT at all times to prevent accidental contamination.
6. The tester will label the tubes. BSL-2 safety practices will be maintained throughout the processing and testing steps.
7. Opening Sample Tubes –
  - a. The TT takes a disinfectant wipe, places it around the stopper at the top of the sample tube for chemistry testing and gently and carefully rocks the stopper off.
  - b. The stopper is placed upon a wipe laid out in the rear of the BSC so that it will not come in contact with the BSC working surface.
  - c. Then place the tube into the rack in the BSC and wipe the external lip of the tube with a disinfectant wipe. Immediately carefully place the wipe into the waste bag.
8. To obtain blood for Malaria testing, refer to section F below.

## F. Testing

1. Chemistry - Perform only with Lab Director approval, refer to the Ebola Processing Checklist (see Related Documents)
2. Hematology and Coagulation - Refer to the specific sections in the Ebola Processing Checklist (see Related Documents)
3. Malaria Rapid Screen -
  - a. Remove one test device for the kit and allow to come to room temperature.
  - b. Prepare the external positive and negative controls.
  - c. Remove test devices from pouch just prior to use. Open the device and lay it flat on the work surface.
  - d. Perform the external controls first, then the test the patient sample.
  - e. The tester slowly adds 15  $\mu$ L of blood to the bottom half of the PURPLE sample pad.
  - f. There is a white pad immediately below the purple sample pad. Hold the Reagent A bottle vertically and add two (2) free-falling drops of Reagent A to this white pad. Allow the first drop to absorb into the pad before adding the second drop. Do not add Reagent A directly to the purple pad.
  - g. Allow the blood sample to run up the full length of the test strip. Do not allow the blood to run into or under the absorbent pad at the top of the strip, as doing so will hinder optimal washing (clearance) of the test strip. Note: If blood flow up the test strip appears to stall or is less than halfway up the strip after one (1) minute, add one (1) additional drop of Reagent A to the white pad at the bottom of the test strip (below the sample pad where the blood was added).
  - h. Just before the blood sample reaches the base of the white absorbent pad located at the top of the test strip, SLOWLY add four (4) free-falling drops of Reagent A to the wash pad on the top left-hand side of the test device, allowing each drop to absorb into the pad before adding the next. Note that the third and fourth drops may not completely absorb into the pad.
  - i. When the sample just reaches the base of the white absorbent pad at the top of the test strip, remove the adhesive liner from the right edge of the device, and close the device. This allows the Reagent A to wash (clear) the blood sample off the test strip. To ensure good device closure and test flow, press very firmly along the entire edge to the right of the result window.
  - j. Read the test result through the viewing window 15 minutes after closing the test device. Results read before or after 15 minutes may be inaccurate. Note: When reading test results, tilt the device to reduce glare on the result window, if necessary.
  - k. Record the external and internal control results and the patient results on the QC /result form.
4. Malaria Smears – THIN PREP only to be done if the rapid malaria test is negative
  - a. Slides will be made, using the CBC lavender top (EDTA) blood tube, inside the BSC.
  - b. The AT labels glass slides outside the BSC and drop- passes (without touching) the slides to the TT.
  - c. The TT uses an EPA registered disinfectant and sprays a 4x4 gauze pad. Using this pad, the TT covers the open end of a DIFF-Safe<sup>®</sup> Blood Dispenser and carefully and gently inserts the device into the EDTA tube.



- d. The TT prepares two (2) thin prep slides for malaria evaluation.
  - e. The TT wipes the exterior of the tube and DIFF-Safe device with another EPA registered disinfectant wipe. The device and the EDTA tube will remain connected.
  - f. The tester allows the slides to air dry within the BSC.
  - g. The tester uses a disposable forceps to place the air-dried slides into a 50 mL conical tube that contains methanol, and caps the lid securely to the tube. Be sure that the all surfaces of the slide are immersed in the methanol.
  - h. The slides will fix in methanol for 30 minutes. After the 30 minute methanol fix, the TT removes the slides using a disposable forceps and allows the malaria slides to air dry.
  - i. When dry, the TT uses a disposable forceps to place the slides in a clean 50-mL conical tube. The TT caps the tube and moves it to the microbiology BSC.
  - j. The TT uses a disposable forceps to place the slides in the slide warmer to heat fix for 60 minutes.
  - k. Using disposable forceps, the TT moves aside while the AT carefully reaches into the BSC without touching any surface of the BSC with his/her PPE and takes the slide off of the heating block.
  - l. The slides will be stained in the automated hematology slide stainer.
  - m. The AT instructs staff when the slides are ready for reading and interpretation.
  - n. All reports will include a comment that indicates that “Only thin smears were evaluated for this test.”
5. Reporting Results
- a. The TT will read the testing results to the AT who will write the results onto a work sheet with the patient’s name, date and time.
  - b. The AT will read the results back to the TT who will verify them.
  - c. Once verified, the AT will enter the results into the LIS and double check them against the work sheet. Once checked, the results will be released to the floors.

## **G. Post Testing**

1. Decontamination of BSC
  - a. TT disposes of any remaining waste into the designated container.
  - b. TT returns all remaining sample tubes to the original transport container and secures the lid.
  - c. TT decontaminates the surfaces of the testing equipment and BSC.
2. Doff the PPE
  - a. Following completion of BSC decontamination, the TT decontaminates their gloves by using a wipe or bleach immersion or spray and seals the waste bag. This will be left in the BSC until the patient is ruled in or ruled out as having EVD.
  - b. The TT and AT carefully make their way to the doffing area without touching anyone or anything in the lab. Accidental contact must be decontaminated.
  - c. Using the Buddy system, the AT and TT help each other doff their equipment onto a carefully placed clean floor covering that will be used to wrap the discarded equipment. Refer to the doffing PPE procedure in Addendum D.

## H. Specimen Storage

Dispose of all **suspected** Ebola specimens upon completion of testing, into the biological hazard trash located in the BSC.

If Ebola virus has been confirmed by appropriate testing, the waste bag containing discarded samples must be handled carefully and in the following manner:

- Working in the BSC and using the PPE as described above, carefully take the red bag waste container and place it into another red bag held open by a second person who also is in the PPE as described above.
- The person placing the first bag into the second must then decontaminate their outer gloves and discard them.
- The second person seals the second bag and places it into a cardboard container which is turned over to the hospital for appropriate disposal as defined by the State of Maryland.
- Any specimens that are confirmed for EVD and contain live-infectious Ebola virus must be reported to the Division of Select Agents and Toxins immediately by telephone (404-718-2000) and be followed up with APHIS/CDC Form 4 within 7 days of the initial report.

## I. Laboratory Safety

All laboratory specimens are handled with standard precautions regardless of the diagnosis. Activities that generate aerosols should be conducted in a Class II Biological Safety Cabinet.

Work surfaces must be decontaminated with appropriate EPA-registered hospital disinfectants upon completion of work. Enveloped viruses such as Ebola are susceptible to a broad range of hospital disinfectants. However the current CDC guideline is to disinfect hard, non-porous surfaces, with Sodium hypochlorite (e.g. 10% bleach), methyl alcohol, phenolic or glutaraldehyde disinfectants, and other EPA-registered hospital disinfectants with label claims against non-enveloped viruses (e.g., norovirus, rotavirus, adenovirus, poliovirus) may be used. Follow the manufacturer's recommendations for use, including dilution, contact time and care in handling.

Waste generated during laboratory testing should be placed in leak-proof containment and discarded as regulated medical waste. For equipment that drains directly into the sewer system, the United States sanitary sewer system handling processes (e.g., anaerobic digestion, composting, disinfection) are designed to safely inactivate infectious agents.

Packaging a specimen for transport from a possible or known case of Ebola viral disease must take place under a BSC. Contact your state and/or local health department and CDC (770-488-7100) to determine the proper category for shipment based on clinical history and risk assessment by CDC and to obtain detailed shipping guidance and required CDC submission documents. State guidelines may differ and state or local health departments should be consulted before shipping.

Local or state public health laboratories and the CDC must be notified immediately when transporting a specimen suspected of Ebola.

**6. RECORDS MAINTENANCE**  
 Not applicable

**7. RELATED DOCUMENTS**

- Quest Diagnostics Bloodborne Pathogens Exposure Control Plan, (QDEHS701); [http://questnet1.qdx.com/Employee\\_Center/environment\\_health\\_safety/ehs\\_toc.htm](http://questnet1.qdx.com/Employee_Center/environment_health_safety/ehs_toc.htm)
- Quest Diagnostics Comprehensive Microbiology Safety Procedures, (QDMI726); [http://questnet1.qdx.com/Business\\_Groups/test\\_the\\_specimen/national\\_testing\\_operations/microbiology/qdmi726.doc](http://questnet1.qdx.com/Business_Groups/test_the_specimen/national_testing_operations/microbiology/qdmi726.doc)
- Quest Diagnostics Safe Transportation of Diagnostic Specimens and Other Hazardous Materials Procedures; [http://questnet1.qdx.com/Business\\_Groups/acquire/logistics/ehs\\_hazmat/HazMat\\_toc.htm](http://questnet1.qdx.com/Business_Groups/acquire/logistics/ehs_hazmat/HazMat_toc.htm)
- Quest Diagnostics Clinical Specimens of High Virulence Organisms (QDMI815)
- Ebola Processing Checklist (AG.F316)

**8. REFERENCES**

- Quest Diagnostics Clinical Specimens of High Virulence Organisms (QDMI815)
- <http://www.cdc.gov/vhf/ebola/pdf/pppe-poster.pdf>

**9. DOCUMENT HISTORY**

Version	Date	Reason for Revision	Revised By	Approved By

**10. ADDENDA**

Addendum	Title
Addendum A	CDC’s Biosafety in Microbiological and Biomedical Laboratories (BMBL), Biosafety Level 2 Practices
Addendum B	CDC’s Biosafety in Microbiological and Biomedical Laboratories (BMBL), Biosafety Level 3 Practices
Addendum C	Donning PPE
Addendum D	Doffing PPE

## Addendum A

### **CDC's Biosafety in Microbiological and Biomedical Laboratories (BMBL) Biosafety Level 2**

Biosafety Level 2 builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that: 1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2) access to the laboratory is restricted when work is being conducted; and 3) all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

The following standard and special practices, safety equipment, and facility requirements apply to BSL-2.

#### **A. Standard Microbiological Practices**

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:
  - a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
  - b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
  - c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
  - d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.

7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:
  - a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
  - b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include: the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.
10. An effective integrated pest management program is required.
  - a. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures.
  - b. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

### ***B. Special Practices***

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
2. Laboratory personnel must be provided medical surveillance, as appropriate, and offered available immunizations for agents handled or potentially present in the laboratory.
3. Each institution should consider the need for collection and storage of serum samples from at-risk personnel.
4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.

6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
  - a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
  - b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
9. Animal and plants not associated with the work being performed must not be permitted in the laboratory.
10. All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.

***C. Safety Equipment (Primary Barriers and Personal Protective Equipment)***

1. Properly maintained BSCs, other appropriate personal protective equipment, or other physical containment devices must be used whenever:
  - a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.
  - b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.
2. Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials. Remove protective clothing before leaving for non-laboratory areas, e.g., cafeteria, library, and administrative offices). Dispose of protective clothing appropriately, or deposit it for laundering by the institution. It is recommended that laboratory clothing not be taken home.
3. Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device. Eye and face

protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories should also wear eye protection.

4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-2 laboratory workers should:
  - a. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
  - b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
  - c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
5. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

#### ***D. Laboratory Facilities (Secondary Barriers)***

1. Laboratory doors should be self-closing and have locks in accordance with the institutional policies.
2. Laboratories must have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It should be located near the exit door.
3. The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.
4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
  - a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
  - b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
5. Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens.
6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Vacuum lines should be protected with liquid disinfectant traps.

8. An eyewash station must be readily available.
9. There are no specific requirements for ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.
10. HEPA filtered exhaust air from a Class II BSC can be safely recirculation back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly exhausted to the outside through a hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.
11. A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).



## Addendum B

### CDC's Biosafety in Microbiological and Biomedical Laboratories (BMBL) Biosafety Level 3 Practices

#### A. *Standard Microbiological Practices*

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.
7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method). Depending on where the decontamination will be performed, the following methods should be used prior to transport:
  - a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
  - b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.
10. An effective integrated pest management program is required. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be

encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

### ***B. Special Practices***

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
2. Laboratory personnel must be provided medical surveillance and offered appropriate immunizations, when available, for agents handled or potentially present in the lab.
3. Each institution should consider the need for collection and storage of serum samples from at-risk personnel.
4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-3 agents.
6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
  - a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
  - b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.
10. All procedures involving the manipulation of infectious materials must be conducted within a BSC, or other physical containment devices. No work with open vessels is conducted on the bench. When a procedure cannot be performed within a BSC, a combination of personal protective equipment and other containment devices, such as a centrifuge safety cup or sealed rotor must be used.

Source: [http://www.cdc.gov/biosafety/publications/bmb15/BMBL5\\_sect\\_IV.pdf](http://www.cdc.gov/biosafety/publications/bmb15/BMBL5_sect_IV.pdf)

## Addendum C

## Donning PPE

### Donning PPE, N95 Respirator Option

- 1 **Engage Trained Observer:** The donning process is conducted under the guidance and supervision of a trained observer who confirms visually that all PPE is serviceable and has been donned successfully. The trained observer will use a written checklist to confirm each step in donning PPE and can assist with ensuring and verifying the integrity of the ensemble. No exposed skin or hair of the healthcare worker should be visible at the conclusion of the donning process.
- 2 **Remove Personal Clothing and Items:** Change into surgical scrubs in a suitable, clean area. No personal items (e.g., jewelry, watches, cell phones, pagers, pens) should be brought into the contaminated area.
- 3 **Inspect PPE Prior to Donning:** Visually inspect the PPE ensemble to be worn to ensure it is in serviceable condition, all required PPE and supplies are available, and that the sizes selected are correct for the healthcare worker. The trained observer reviews the donning sequence with the healthcare worker before the healthcare worker begins and reads it to the healthcare worker in a step-by-step fashion.
- 4 **Perform Hand Hygiene:** Perform hand hygiene with ABHR\*. When using ABHR, allow hands to dry before moving to next step.
- 5 **Put on Inner Gloves:** Put on first pair of gloves. (surgical gloves)
- 6 **Put on Shoe Covers.**
- 7 **Put on Gown:** Put on gown. Ensure gown is large enough to allow unrestricted freedom of movement. Ensure cuffs of inner gloves are tucked under the sleeve of the gown.
- 8 **Put on N95 Respirator:** Put on N95 respirator. Complete a user seal check.
- 9 **Put on Surgical Hood:** Over the N95 respirator, place a surgical hood that covers all of the hair and the ears, and ensure that it extends past the neck to the shoulders. Be certain that hood completely covers the ears and neck.
- 10 **Put on Outer Gloves:** Put on second pair of gloves (standard lab gloves). Ensure the cuffs are pulled over the sleeves of the gown.
- 11 **Put on Face Shield:** Put on full face shield over the N95 respirator and surgical hood to provide additional protection to the front and sides of the face, including skin and eyes.
- 12 **Verify:** After completing the donning process, the integrity of the ensemble is verified by the trained observer. The healthcare worker should be comfortable and able to extend the arms, bend at the waist and go through a range of motions to ensure there is sufficient range of movement while all areas of the body remain covered. A mirror in the room can be useful for the healthcare worker while donning PPE.
- 13 **Disinfect Outer Gloves:** Disinfect outer-gloved hands with ABHR. Allow to dry prior to beginning work.

\*ABHR: alcohol-based hand rub

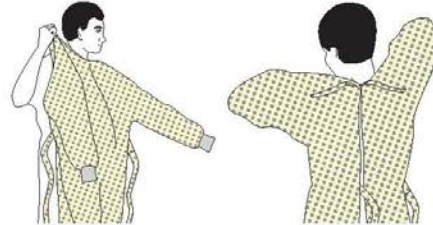
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## SEQUENCE FOR PUTTING ON PERSONAL PROTECTIVE EQUIPMENT (PPE)

The type of PPE used will vary based on the level of precautions required, such as standard and contact, droplet or airborne infection isolation precautions. The procedure for putting on and removing PPE should be tailored to the specific type of PPE.

### 1. GOWN

- Fully cover torso from neck to knees, arms to end of wrists, and wrap around the back
- Fasten in back of neck and waist



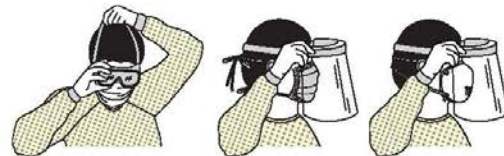
### 2. MASK OR RESPIRATOR

- Secure ties or elastic bands at middle of head and neck
- Fit flexible band to nose bridge
- Fit snug to face and below chin
- Fit-check respirator



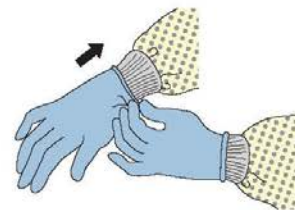
### 3. GOGGLES OR FACE SHIELD

- Place over face and eyes and adjust to fit



### 4. GLOVES

- Extend to cover wrist of isolation gown



## USE SAFE WORK PRACTICES TO PROTECT YOURSELF AND LIMIT THE SPREAD OF CONTAMINATION

- Keep hands away from face
- Limit surfaces touched
- Change gloves when torn or heavily contaminated
- Perform hand hygiene



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## Addendum D

## Doffing PPE

### Doffing PPE, N95 Respirator Option

PPE doffing is performed in the designated PPE removal area. Place all PPE waste in a leak-proof infectious waste container (<http://www.cdc.gov/vhf/ebola/hcp/environmental-infection-control-in-hospitals.html>).

- 1 **Engage Trained Observer:** The doffing process is conducted under the supervision of a trained observer, who reads aloud each step of the procedure and confirms visually that the PPE has been removed properly. Prior to doffing PPE, the trained observer must remind healthcare worker to avoid reflexive actions that may put them at risk, such as touching their face. Post this instruction and repeat it verbally during doffing. Although the trained observer should minimize touching healthcare workers or their PPE during the doffing process, the trained observer may assist with removal of specific components of PPE as outlined below. The trained observer disinfects the outer-gloved hands immediately after handling any healthcare worker PPE.
- 2 **Inspect:** Inspect the PPE to assess for visible contamination, cuts, or tears before starting to remove. If any PPE is visibly contaminated, then disinfect using an \*EPA-registered disinfectant wipe.
- 3 **Disinfect Outer Gloves:** Disinfect outer-gloved hands with an \*EPA-registered disinfectant wipe.
- 4 **Remove Shoe Covers:** Remove and discard shoe covers.
- 5 **Disinfect and Remove Outer Gloves:** Disinfect outer-gloved hands with an \*EPA-registered disinfectant wipe. Remove and discard outer gloves taking care not to contaminate inner gloves during removal process.
- 6 **Inspect and Disinfect Inner Gloves:** Inspect the inner gloves' outer surfaces for visible contamination, cuts, or tears. If an inner glove is visibly soiled, cut, or torn, then disinfect the glove with either an \*EPA-registered disinfectant wipe. Then remove the inner gloves, perform hand hygiene with ABHR on bare hands, and don a clean pair of gloves. If visible contamination, cuts, or tears are identified on the inner gloves, then disinfect the inner-gloved hands with either an \*EPA-registered disinfectant wipe.
- 7 **Remove Face Shield:** Remove the full face shield by tilting the head slightly forward, grabbing the rear strap and pulling it over the head, gently allowing the face shield to fall forward and discard. Avoid touching the front surface of the face shield.
- 8 **Disinfect Inner Gloves:** Disinfect inner gloves with either an \*EPA-registered disinfectant wipe.
- 9 **Remove Surgical Hood:** Unfasten (if applicable) surgical hood, gently remove, and discard. The trained observer may assist with unfastening the hood.
- 10 **Disinfect Inner Gloves:** Disinfect inner gloves with an \*EPA-registered disinfectant wipe.
- 11 **Remove Gown:** Remove and discard.  
Depending on gown design and location of fasteners, the healthcare worker can either untie fasteners, receive assistance by the trained observer to unfasten to gown, or gently break fasteners. Avoid contact of scrubs with outer surface of gown during removal. Pull gown away from body, rolling inside out and touching only the inside of the gown.
- 12 **Disinfect and Change Inner Gloves:** Disinfect inner gloves with an \*EPA-registered disinfectant wipe. Remove and discard gloves taking care not to contaminate bare hands during removal process. Perform hand hygiene with ABHR. Don a new pair of inner gloves.
- 13 **Remove N95 Respirator:** Remove the N95 respirator by tilting the head slightly forward, grasping first the bottom tie or elastic strap, then the top tie or elastic strap, and remove without touching the front of the N95 respirator. Discard N95 respirator.
- 14 **Disinfect Inner Gloves:** Disinfect inner gloves with either an \*EPA-registered disinfectant wipe.

- 15 **Disinfect and Remove Inner Gloves:** Disinfect inner-gloved hands with an \*EPA-registered disinfectant wipe. Remove and discard gloves taking care not to contaminate bare hands during removal process.
- 16 **Inspect:** Perform a final inspection of healthcare worker for any indication of contamination of the surgical scrubs. If contamination is identified, immediately inform infection preventionist or occupational safety and health coordinator or their designee before exiting PPE removal area.

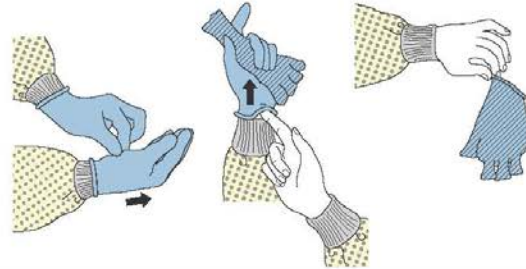
\*EPA-registered disinfectant wipe: Use a disposable wipe impregnated with a U.S. Environmental Protection Agency (EPA)-registered hospital disinfectant with a label claim of potency at least equivalent to that for a non-enveloped virus (e.g., norovirus, rotavirus, adenovirus, poliovirus).  
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## HOW TO SAFELY REMOVE PERSONAL PROTECTIVE EQUIPMENT (PPE) EXAMPLE 1

There are a variety of ways to safely remove PPE without contaminating your clothing, skin, or mucous membranes with potentially infectious materials. Here is one example. **Remove all PPE before exiting the patient room** except a respirator, if worn. Remove the respirator **after** leaving the patient room and closing the door. Remove PPE in the following sequence:

### 1. GLOVES

- Outside of gloves are contaminated!
- If your hands get contaminated during glove removal, immediately wash your hands or use an alcohol-based hand sanitizer
- Using a gloved hand, grasp the palm area of the other gloved hand and peel off first glove
- Hold removed glove in gloved hand
- Slide fingers of ungloved hand under remaining glove at wrist and peel off second glove over first glove
- Discard gloves in a waste container



### 2. GOGGLES OR FACE SHIELD

- Outside of goggles or face shield are contaminated!
- If your hands get contaminated during goggle or face shield removal, immediately wash your hands or use an alcohol-based hand sanitizer
- Remove goggles or face shield from the back by lifting head band or ear pieces
- If the item is reusable, place in designated receptacle for reprocessing. Otherwise, discard in a waste container



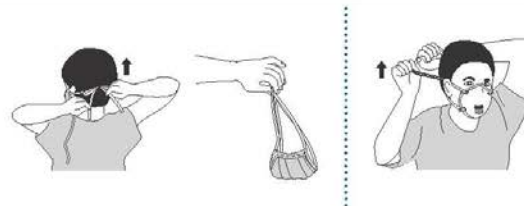
### 3. GOWN

- Gown front and sleeves are contaminated!
- If your hands get contaminated during gown removal, immediately wash your hands or use an alcohol-based hand sanitizer
- Unfasten gown ties, taking care that sleeves don't contact your body when reaching for ties
- Pull gown away from neck and shoulders, touching inside of gown only
- Turn gown inside out
- Fold or roll into a bundle and discard in a waste container

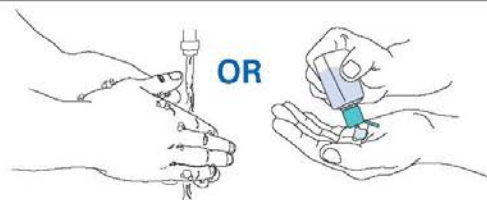


### 4. MASK OR RESPIRATOR

- Front of mask/respirator is contaminated — **DO NOT TOUCH!**
- If your hands get contaminated during mask/respirator removal, immediately wash your hands or use an alcohol-based hand sanitizer
- Grasp bottom ties or elastics of the mask/respirator, then the ones at the top, and remove without touching the front
- Discard in a waste container



### 5. WASH HANDS OR USE AN ALCOHOL-BASED HAND SANITIZER IMMEDIATELY AFTER REMOVING ALL PPE



**PERFORM HAND HYGIENE BETWEEN STEPS IF HANDS  
BECOME CONTAMINATED AND IMMEDIATELY AFTER  
REMOVING ALL PPE**



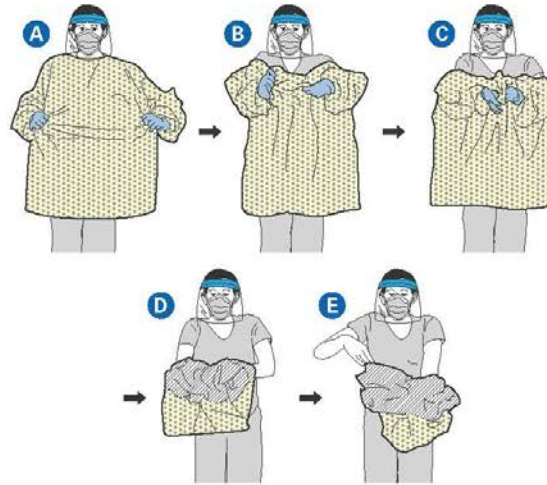
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## HOW TO SAFELY REMOVE PERSONAL PROTECTIVE EQUIPMENT (PPE) EXAMPLE 2

Here is another way to safely remove PPE without contaminating your clothing, skin, or mucous membranes with potentially infectious materials. **Remove all PPE before exiting the patient room** except a respirator, if worn. Remove the respirator **after** leaving the patient room and closing the door. Remove PPE in the following sequence:

### 1. GOWN AND GLOVES

- Gown front and sleeves and the outside of gloves are contaminated!
- If your hands get contaminated during gown or glove removal, immediately wash your hands or use an alcohol-based hand sanitizer
- Grasp the gown in the front and pull away from your body so that the ties break, touching outside of gown only with gloved hands
- While removing the gown, fold or roll the gown inside-out into a bundle
- As you are removing the gown, peel off your gloves at the same time, only touching the inside of the gloves and gown with your bare hands. Place the gown and gloves into a waste container



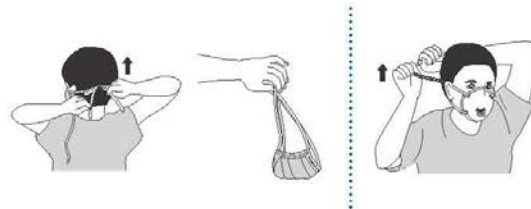
### 2. GOGGLES OR FACE SHIELD

- Outside of goggles or face shield are contaminated!
- If your hands get contaminated during goggle or face shield removal, immediately wash your hands or use an alcohol-based hand sanitizer
- Remove goggles or face shield from the back by lifting head band and without touching the front of the goggles or face shield
- If the item is reusable, place in designated receptacle for reprocessing. Otherwise, discard in a waste container

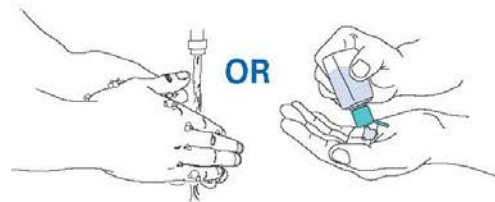


### 3. MASK OR RESPIRATOR

- Front of mask/respirator is contaminated — DO NOT TOUCH!
- If your hands get contaminated during mask/respirator removal, immediately wash your hands or use an alcohol-based hand sanitizer
- Grasp bottom ties or elastics of the mask/respirator, then the ones at the top, and remove without touching the front
- Discard in a waste container



### 4. WASH HANDS OR USE AN ALCOHOL-BASED HAND SANITIZER IMMEDIATELY AFTER REMOVING ALL PPE



**PERFORM HAND HYGIENE BETWEEN STEPS IF HANDS  
BECOME CONTAMINATED AND IMMEDIATELY AFTER  
REMOVING ALL PPE**



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