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Microbiology Procedure for Handling and Processing of Specimens from Patients with Suspected or Documented Viral Hemorrhagic Fever (VHF)



A. Notification and Approval of VHF Testing

1. When a patient with suspect VHF is identified:
 - a. the treating team will:
 - i. notify the Microbiologist-on-call immediately
 - ii. share the patient's clinical information
 - b. the Microbiologist-on-call will:
 - i. collect clinical and epidemiologic information using the [Viral Hemorrhagic Fever \(VHF\) Epidemiologic Tool](#) and [Assessing risk of EVD in ED patients](#) as guidance
 - ii. **For Mount Sinai Hospital (MSH) Patients:**
 - the Microbiologist-on-call should follow [the ED communications flowchart](#) or the [outpatient area communications flowchart](#) for direction of their involvement
 - c. **If the laboratory is not notified ahead of time, and specimens from patients with suspect or documented VHF are already in the lab when the lab is notified of the suspicion for Ebola, then the specimen(s) or the tests/cultures related to them should not be handled and the Microbiologist-on-call should be notified immediately** in order to instruct the lab on how to handle the specimen(s) and test(s) already in circulation and how to decontaminate the lab and those who handled the specimen(s) or test(s)
2. If VHF testing is deemed warranted:
 - a. the Microbiologist-on-call will:
 - i. contact Public Health Ontario Laboratory (PHOL) (Customer Service **(416-235-6556 or 1-877-604-4567)** Mon-Fri 0730-1900, Sat 0800-1545 OR the **PHOL Duty Officer after-hours at 416-605-3113**) to notify them of the request for VHF testing and malaria if patient is referred from a clinic, see Appendix A for handling process

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- if testing is approved, PHOL will provide shipping details including the PHOL Toronto address where the specimen should be shipped, the name of the recipient at PHOL and a PHOL phone number that will be answered by a person with knowledge of the shipment if there is a problem
- the microbiologist-on-call should assure that the shipping document is updated as necessary to reflect this information

3. If VHF testing is approved by PHOL:

a. the Microbiologist-on-call will:

i. contact the National Microbiology Laboratory (NML) Operations Center Director – OCD at 1-8660262-8433) to:

- update them that Ebola testing has been approved at PHOL (PHOL will already have alerted NML of this);
- ask that NML put the Health Canada/Transport Canada approved Emergency Response Activation Plan (ERAP) for transportation of Ebola related specimens on stand-by; and
- confirm that the ERAP and CANUTEC information on the shipping document is accurate
- Note: NML needs to be notified and ERAP needs to be on stand-by when specimens are being transported by vehicle or foot between hospital sites or between hospitals and PHOL; this does not apply when specimens are being transported within the same hospital building or through hospital tunnels from one hospital site to another hospital site

ii. follow the relevant hospital's protocol:

- **For University Health Network (UHN) specimens arriving from the Toronto Western Hospital (TWH):**

a. contact the UHN manager-on-call (14-LABS/14-5227 press 1) to:



i. update them of the upcoming specimens

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- ii. confirm that the Health Canada ERAP has been put on standby
- iii. activate specimen management's role in arranging for direct observation of specimen collection and packaging and arranging LifeLabs transportation of the specimens to PHOL, Toronto General Hospital Core Lab, and University Health Network/Mount Sinai Hospital Microbiology Laboratory according to Transportation of Dangerous Goods Category A shipment
- **For MSH Patients:**
 - a. Mount Sinai is not designated as a testing or treating hospital for suspect EVD patients but the Microbiologist-on-call will be asked to assess the patient to determine the need for testing
 - b. If testing is needed, the attending of the patient unit should call Crite-Call to arrange the patient to be transferred to a testing hospital
 - c. While waiting to be transferred:
 - **For patients in the Emergency Department (ED) (see ED communications flowchart):**
 - i. Contact Infection Control (refer to Infection Control phone numbers provided in the monthly Microbiologists' call schedule) to request two ICPs to attend to the emergency department (ED) to:
 - Notify housekeeping; identify patient points of contact; direct cleaning schedule (ICP#1)
 - Act as a trained observer for personal protective equipment (PPE) and patient care (ICP#2)



For patients in an Outpatient Area (see outpatient communications flowchart):

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- ii. Contact Infection Control (refer to Infection Control phone numbers provided in the monthly Microbiologists' call schedule) to request two ICPs to:
 - Go to the outpatient area; notify housekeeping; identify patient points of contact; direct cleaning schedule (ICP#1)
- iii. If it is decided that the patient should be sent to ED for isolation while waiting to be transported to a testing hospital, then also:
 - Request two ICPs prepare the ED team to prepare Resus. Room 4 and don PPE; notify security to plan a transport route to the ED from the outpatient location; act as a trained observer PPE and patient care (ICP#2)
 - Contact the ED Duty Physician in Major to update him of the patient and the impending transfer of the patient from the outpatient area to the ED.

For all patients, regardless of location:



- iv. Update the hospital administrator-on-call who will notify all relevant heads of department and administrative heads through the MSH Ebola Fan-Out List email distribution list
 - **For specimens arriving from sites other than Mount Sinai Hospital and UHN sites other than TWH:**
 - a. contact the site's laboratory director (through the site's locating) to discuss specimen transportation arrangements and provide the ERAP number
- iii. **contact Toronto Public Health** (TPH Ebola Information Line for Health Care Professionals at 416-392-5311 during regular business hours or 311

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after hours) **and the patient's resident address local public health unit ([Reportable Diseases to the Medical Office Health QPCMI16001](#))**

- report the suspect case
- iv. **notify the Medical Microbiologist-in-chief Dr. Tony Mazzulli, director Kit Wong, and supervisor Pauline Lo** of the UHN/MSH Department of Microbiology via email (tmazzulli@mtsinai.on.ca; kwong@mtsinai.on.ca, plo@mtsinai.on.ca) to:
 - notify them of the suspect case
- v. **contact the Microbiology Laboratory Staff**
 - during regular hours: weekdays 0800-2300; weekends and holidays 0800-1600 through 416-586-4432 option 2, contact the **Designated Senior and assign an assistant and assure a senior laboratory person acting as supervisor is present** to observe the Designated Senior and assistant or
 - after-hours **activate the Staff FAN OUT list and call in appropriate staff** using the [MSH Staff Fan out List](#) to:
 - a. notify them of the anticipated specimens
 - b. remind them that they will initiate and be responsible for completing the [VHF Specimens Handling Log](#) sheet

B. Preparation for Arrival of Specimens from Patients with Suspected or Documented VHF


1. Only the Designated Senior, the assigned assistant, the Microbiologist(s) overseeing the case, and the laboratory supervisor/director should be involved in the processing of these specimens. Only the Designated Senior and assistant should work in the "Level 2.5 laboratory"/anteroom. All others should be in the adjacent hallway.
2. The Designated Senior will:
 - I. Print [VHF Specimens Handling Log](#) and be responsible for ensuring the log is filled by all staff handling the specimens of the case

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- II. Electronically receive and print specimen and requisition labels for the anticipated specimens in the LIS, i.e.
- i. **2 blood culture sets (4 bottles)** (to be processed in UHN/MSH Department of Microbiology)

Note: Labels for 3 EDTA tubes for VHF detection and malaria detection will be printed by the ward. PHOL requisition will also be printed by the ward and specimens directly shipped to PHOL following **Class 6.2 Category A (UN2814) shipping procedures.**



III. **Prepare the “Level 2.5” Laboratory:**

- a. retrieve the [VHF PPE Kit](#) and [VHF Specimen Kit](#) (from the Spill Kits area) and bring it on the trolley to the “Level 2.5” laboratory (designated as the **VHF trolley**)
- b. place the “**DO NOT ENTER** – Ebola Processing in Progress” sign on the door to the “Level 2.5” Laboratory
- c. assure that the “Level 2.5” laboratory has:
 - i. one sharps cardboard box lined with two autoclave bags;
 - ii. long cuff nitrile gloves of the right size for the Designated Senior;
 - 10 pairs beside the BSC on the counter “dirty side”
 - 2 pairs on “clean side”
 - iii. Virox wipes container
 - iv. Liquid virox
 - v. alcohol sanitizer
 - vi. shipping tape
 - vii. 2 face shields on the clean side of the bench
- d. assure that the anteroom has:
 - i. one sharps cardboard box lined with two autoclave bags;
 - ii. glove of the right size for the assistant;
 - iii. Virox wipes container
 - iv. alcohol sanitizer
- e. clear the “Level 2.5” laboratory of any non-essential items

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- f. turn on the Biosafety cabinet (BSC) and check the airflow gauge to ensure that it is running properly
- g. open the [VHF Specimen Kit](#) and prepare the BSC work area and supplies as follows:
 - ii. clean the BSC using Virox wipes; keep the Virox wipes in the BSC
 - iii. lay two blue pads in the clean BSC, one to be used for placing the specimens prior to re-cleaning with Virox wipes (“dirty blue pad”) and one for use after re-cleaning with Virox wipes (“clean blue pad”)
 - iv. place the following onto the **“dirty blue pad”**:
 1. disposable waste container
 - v. place the following onto the **“clean blue pad”**:
 1. LIS labels
 2. clean plastic specimen bags,

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IV. Prepare the Anteroom of the “Level 2.5” Laboratory

- a. obtain a new trolley and prepare it to be the **BC transport trolley** as follows:
 - i. place the following items on the top shelf of the trolley
 1. blue pad
 2. Opened hard plastic shipping container
 3. alcohol sanitizer
 - ii. place the following items on the middle shelf of the trolley
 1. gloves
 - iii. place the trolley in the anteroom outside of the “Level 2.5” laboratory to keep these items clean

C. Receiving Specimens from Patients with Suspected or Documented VHF

1. When the specimens arrive in the laboratory, transfer the specimen from the porter to the level 2.5 lab’s BSC.
2. Specimens will be packaged inside a clean hard plastic specimen transport container that will be packaged inside a Category A cardboard shipping box and transported by courier service to Microbiology following Category A Transportation of Dangerous Good Regulations

D. Don PPE:

For both the designated senior and the assistant:

- a. put on a standard lab coat
- b. don PPE using supplies from the [VHF PPE Kit](#) following the order in the [Donning/Doffing VHF PPE appendix](#) with the assistant observing and confirming that donning was completed correctly

E. Preparation of Blood Cultures in the “Level 2.5” Laboratory



1. The Designated Senior will:
 - a. open the shipping containers
 - b. remove the blood culture bottles from the hard plastic specimen container inside the BSC and place them on the “dirty blue pad”

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- c. remove the blood culture bottles from their biosafety bag and wipe the bottles including the tops with a Virox wipe and place them on the “clean blue pad”
- d. remove outer layer of gloves and replace with new ones
- e. attach LIS labels on the bottles and place a small LIS label on the ring over the septum of each bottle and place them into a new plastic biosafety specimen bag
- f. wipe the outside of the biosafety specimen bag with a Virox wipe and seal the bag
- g. remove outer layer of gloves and replace with new ones
- h. open the door and place the biosafety bag containing the blood culture bottles into the open hard plastic shipping container on the blue pad on top of the **BC transport trolley**

F. Incubating Blood Cultures



1. The assistant will:
 - a. close the hard plastic shipping container
 - b. remove outer gloves, replace with a new set
 - c. perform hand hygiene
 - d. transport the **BC transport trolley** to the blood culture incubator
 - e. once at the blood culture incubator, open the designated VHF blood culture drawer
 - f. don two pairs of gloves
 - g. remove the blood culture bottles from the hard plastic shipping container and biosafety bag using the trolley as a workstation
 - h. hold the blood culture bottles for scanning careful not to touch the scanner
 - i. place the blood culture bottles in the designated VHF slots in the incubator taking care not to touch anything but the blood culture bottles
 - j. remove gloves and places them on the blue pad
 - k. perform hand hygiene
 - l. closes the incubator drawer
 - m. brings the **BC transport trolley** back into the anteroom of the “Level 2.5” laboratory
 - n. don two pairs of gloves

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- o. remove the blue pad and specimen bag and gloves from the top of the trolley and place them in the autoclave bag lined sharps box in the anteroom
- p. remove the outer layer of gloves and replace with new ones
- q. wipe down the **BC transport trolley** with liquid Virox and Virox wipes two times keeping it wet for 5 minutes each time
- r. remove gloves
- s. perform hand hygiene
- t. retrieve Ebola waste drum from washup.

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G. Clean Up

1. The Designated Senior will:

I. Clean up the “Level 2.5” laboratory:

- a. take the waste container, transport container and blue pads out of the biosafety cabinet and place them inside the autoclave bag lined sharps box
- b. remove outer layer of gloves and replace with new gloves
- c. clean the “Level 2.5” laboratory carefully with liquid Virox and Virox wipes twice letting it soak for 5 minutes and dry between cleans paying particular attention to:
 - inside of the biosafety cabinet
 - the bench top
 - the Virox wipe container
 - the trolley
 - the courier bag (both inside and outside)
 - high-touch surface of the room (e.g. door knobs)
- d. place unused gloves inside the autoclave bag lined sharps box
- e. tie both layers of the autoclave bag
- f. close the sharps box securely with tape
- g. open the door
- h. place the closed sharps box into the ebola waste drum
- i. bring the anteroom autoclave bag lined sharps box into the “Level 2.5” laboratory

2. Doff PPE:



Designated senior will Doff first, follow by the assistant.

- a. remove all PPE in the “Level 2.5” laboratory **following the order and instructions in the [Donning/Doffing VHF PPE appendix](#)** with the assistant observing and confirming that doffing is being completed correctly. The ungarbed senior will observe the assistant on their turn.
- b. put all doffed PPE into the autoclave bag lined sharps box
- c. perform hand hygiene
- d. go to the anteroom and don two sets of gloves
- e. return to the “Level 2.5” laboratory and put on face shield.

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- f. tape both layers of autoclave bags keeping them within the sharps box
 - g. close the sharps box securely with tape
 - h. wipe the Virox wipe container with a Virox wipe and use the Virox wipe to open the door
 - i. place the sharps box into the drum
- 3. Drum waste:**
- a. Move drum bin to the washup room
 - b. once the VHF status of the patient is known:
 - If VHF has been ruled out – remove the label and place the autoclaved waste sharps box into the bin for routine biological waste disposal (incineration)
 - If VHF has been confirmed or remains unknown – keep the label and discard with instructions from the hospital’s waste management. Call Support Services through Locating (ext. 5133) to request Support Services Staff to supervise packaging of waste for disposal as per
 - Start with small biohazard waste cardboard box, lined with two small red plastic bags
 - Place waste into small red plastic liner, tie off securely
 - Then tie off second red plastic liner securely
 - Then close the small box and tape shut and tape open seams
 - Then place the box into larger red liner
 - The place that package into larger cardboard box and attached the **label UN2814 (update to include newest waste mgt protocol with large plastic discard barrel – suggest put as appendix so don’t have to update multiple spots in SOP)**
- 4. Restocking Supplies, Specimen Handling Log**
- a. Ask the designated safety officer asking him/her to:
 1. restock the lab’s [VHF PPE kit](#) and [VHF Specimen Kit](#)

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- b. once cleaned by Housekeeping, remove the “**DO NOT ENTER**-Ebola Specimen In Progress ” sign on the door to the “Level 2.5” Laboratory
- c. complete the [VHF Specimens Handling Log](#) sheet

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Microbiology Procedure for Handling and Processing Positive Blood Culture Specimens from Patients with Suspected of Confirmed Viral Hemorrhagic Fever (VHF)

A. Confirmation of the Need to Proceed with Subculturing VHF Blood Culture Bottles

1. When a blood culture from a patient with suspect or confirmed Viral Hemorrhagic Fever (VHF) is identified:
 - a. the blood culture technologist will:
 - i. notify the Microbiologist-on-call immediately who will contact the treating team to determine if testing is warranted

B. Preparation for Subculturing VHF Blood Culture Bottles if Testing is Deemed Warranted by the On-Call Microbiologist



1. The Microbiologist-on-call will:
 - a. notify the Designated Senior of the need to process the blood culture bottles
2. The Designated Senior will:
 - a. initiate and be responsible for completing the [VHF Specimens Handling Log](#) sheet
 - b. place the “**DO NOT ENTER**-Ebola Specimen In Progress” sign on the door to the “Level 2.5” Laboratory
 - c. print LIS labels and obtain appropriate agar plates and place them on a trolley
 - d. retrieve the [VHF PPE Kit](#) and [VHF Blood Culture Kit](#) (from the Spill Kits area) and bring it on the trolley to the “Level 2.5” laboratory (designated as the **VHF Positive BC Trolley**)
 - e. assure that the “Level 2.5” laboratory has:
 - i. one large sharps cardboard box lined with two autoclave bags;
 - ii. long cuff nitrile gloves of the right size for the Designated Senior;
 - iii. Virox liquid and wipes;
 - iv. alcohol sanitizer;
 - f. assure that the anteroom has:
 - i. one large sharps cardboard box line with two autoclave bags;
 - ii. glove of the right size for the assistant;
 - iii. Virox wipes, and

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- iv. alcohol sanitizer
- g. clear the “Level 2.5” laboratory of any non-essential items
- h. turn on the Biosafety cabinet (BSC) and check the airflow gauge to ensure that it is running properly
- i. open the [VHF Blood Culture Kit](#) and prepare the biosafety cabinet work area and supplies as follows:
 - i. clean the biosafety cabinet using Virox wipes; keep the Virox wipes in the biosafety cabinet
 - ii. lay two blue pads in the clean biosafety cabinet, one to be used for placing the blood culture bottles (“dirty blue pad”), one for preparing the slides and agar plates (“clean blue pad”)
 - iii. label slide and standard agars prepare QC organisms for CO₂ (*S. pneumoniae* ATCC 49619) and anaerobic incubators (see [Quality Manual- Anaerobic Jar QC organisms](#)) in the main lab
- iv. place the following onto the **“dirty blue pad”**:
 - 1. the sharps container
- v. place the following onto the **“clean blue pad”**:
 - 1. blood culture needles
 - 2. labelled slides and culture plates
 - 3. loops for streaking
 - 4. forceps
 - 5. a biosafety specimen bag
 - 6. the screw top hard plastic transport container (containing an absorbent pad) pre-labelled as “Suspect VHF Blood Cultures – DO NOT TOUCH”
 - 7. 2 snap lid incubator boxes along with one CO₂ and one anaerobic gas pack and two indicator strips
- j. prepare methanol and 10% buffered formalin fixatives:
 - i. contact the Mount Sinai Hospital Core Laboratory in order to coordinate obtaining 10% buffered formalin (Call Histology/Gross Section 4484, 4485); if a core laboratory technologist is not available to dispense 10%

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buffered formalin, then wait for regular day hours when assistance is available before continuing



- ii. prepare the **VHF Positive BC Trolley** as follows:
 1. Place a blue pad on top shelf of the trolley
 2. Place the following on the top shelf of the trolley on top of the blue pad:
 - a. two plastic slide holders, each placed into separate sterile pink top containers (which act as stands keeping the slide holders upright)
 - b. a clean biosafety specimen bag into the hard plastic container such that the specimen bag is kept open and upright
 - c. alcohol sanitizer
 - d. Virox wipes
- iii. bring the **VHF Positive BC Trolley** to the Media Room
- iv. in the BSC, fill one slide holder with absolute methanol
- v. place the slide holder with absolute methanol on the “clean blue pad” in the BSC within the “Level 2.5” laboratory
- vi. bring the **VHF Positive BC trolley** to the Mount Sinai Hospital Histopathology Laboratory (6th floor Room 6-300) and ask for assistance to fill the other slide holder with 10% buffered formalin
- vii. using the **VHF Positive BC trolley**, bring the slide holder with 10% buffered to the “Level 2.5” laboratory and place it on the “clean blue pad”
- k. obtain a second trolley to be used as the **VHF Gram Stain Trolley** and place a blue pad on its top shelf and place it in the anteroom
- l. place the empty [VHF Blood Culture Kit](#) container on the bottom shelf of the **VHF Gram Stain Trolley** in the anteroom
- m. assign an assistant who will help with “clean” tasks
3. The assistant will:
 - a. wear standard lab gear
 - b. stay in the anteroom of the “Level 2.5” laboratory waiting to assist the Designated Senior

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C. Transporting VHF Blood Culture Bottles to the “Level 2.5” Laboratory

1. The Designated Senior will:
 - a. wear standard lab gear
 - b. transport the **VHF Positive BC trolley** to the blood culture incubator
 - c. once at the blood culture incubator, open the designated VHF blood culture drawer
 - d. don two pairs of gloves
 - e. remove the blood culture bottles from the incubator and place them into the upright biosafety specimen bag on the blue pad of the trolley taking care not to touch anything other than the bottles
 - f. remove the outer layer of gloves and replace with new ones
 - g. close the biosafety specimen bag
 - h. remove gloves placing them carefully into the waste container on the trolley
 - i. perform hand hygiene
 - j. close the incubator drawer
 - k. brings the **VHF Positive BC trolley** back into the anteroom of the “Level 2.5” laboratory

D. Subculturing VHF Blood Cultures & Incubating Subcultured Plates



1. The Designated Senior will:
 - a. assigns an assistant who will wear standard lab gear and who will help with “clean” tasks
 - I. **Don PPE:**
 - a. Put on a standard lab coat
 - b. Don PPE using supplies from the [VHF PPE Kit](#) following the order in the [Donning/Doffing VHF PPE appendix](#) with the assistant observing and confirming that donning was completed correctly
 - c. place the empty [VHF PPE Kit](#) container on the bottom shelf of the **VHF Gram Stain Trolley** in the anteroom

II. Subculture Blood Cultures:

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- a. moves the **VHF Positive BC Trolley** into the “Level 2.5” laboratory
- b. place the biosafety bag containing the blood culture bottles from the **VHF Positive BC Trolley** onto the “dirty blue pad” in the BSC
- c. remove blood culture bottles from the specimen bag and wipe them with Virox
- d. prepare blood culture smears and subculture agars as per usual blood culture subculturing procedures taking care not to spill blood or obtain a needlestick injury (see [Blood Culture Manual](#))
- e. remove outer layer of gloves and replace with clean ones
- f. fix blood culture smears as follows:
 - i. allow the smear to air dry within the BSC
- g. while waiting for the slides to air dry, prepare blood culture bottle(s) for storage for possible future use as follows:
 - i. return to the BSC and wipe blood culture bottles with a Virox wipe
 - ii. remove outer layer of gloves and replace with new gloves
 - iii. place blood culture bottles into a clean specimen bag and place this into the screw top hard plastic transport container with absorbent pad prelabelled “**Suspect EBOLA Blood Cultures – DO NOT TOUCH**”
 - iv. wipe the hard plastic container with Virox wipes and place in the corner of the BSC where it will be stored until it is cleared to be discarded after assessment of blood culture subculture plates
- h. place agar plates into snap incubator containers as follows:
 - i. hand streak the plates
 - ii. allow inoculum to dry on agar plates
 - iii. once dry, close agar plates and wipe the outside of each with a Virox wipe
 - iv. carefully place the agar plates and the appropriate QC organism agar plates into the snap incubation containers taking care not to drop the plates
 - v. prepare the CO₂ and anaerobic gas packs
 - vi. wipe gloves with a Virox wipe and let them dry
 - vii. snap close the lids
 - viii. wipe the outside of the snap containers with a Virox wipe
 - ix. wipe gloves with a Virox wipe and let them dry

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- x. remove these from the BSC and place them on the laboratory bench by the incubator in the “Level 2.5” Laboratory
- xi. open the incubator and place the snap containers into the incubator carefully
- xii. label the incubator as “**CONTAINS EBOLA BC CUBCULTURES – DO NOT OPEN**”



E. Gram Staining and Reading Blood Culture Smears

1. The assistant will:
 - a. Move the **VHF Gram Stain Trolley** close to the door of the “Level 2.5” laboratory
2. The Designated Senior will:
 - a. fix the smear with absolute methanol for 30 minutes followed by 10% buffered formalin for 15 minutes using new forceps for each transfer of the slide taking care not to touch the fixed slide with anything but the forceps (references PHAC <http://www.phac-aspc.gc.ca/id-mi/vhf-fvh/ebola-biosafety-biosecureite-eng.php> , ASM <https://www.asm.org/images/PSAB/Ebola9-10-14.pdf>)
 - b. after the smear is fixed, open the door and using new forceps, place the fixed smear on the blue pad of the top shelf of the **VHF Gram Stain Trolley** taking care not to touch anything but the forceps
3. The assistant will:
 - a. wait in the anteroom for the slide to air dry
 - b. transport the slide to the main lab using the **VHF Gram Stain Trolley**
 - c. gram stain and read the smear as per usual protocol (see [Blood Culture Manual](#)).
 - d. discard the blue pad with the usual lab waste
 - e. take the empty [VHF PPE Kit](#) and [VHF Blood Culture Kit](#) containers on the bottom shelf of the **VHF Gram Stain Trolley** to the designated safety officer asking him/her to restock them
 - f. use the **VHF Gram Stain Trolley** to pick up an autoclave bin (from hereon, this trolley is described as the **Autoclave trolley**) and bring it to the anteroom
 - g. place the **Autoclave trolley** and anteroom yellow autoclave bag next to the “Level 2.5” laboratory door

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F. Clean Up



1. Refer to [Clean Up](#) Protocol for Section B. Preparation for Arrival of Specimens from Patients with Suspected or Document VHF (but replace the reference to **VHF Trolley** with **VHF Positive BC Trolley**)

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Microbiology Procedure for Reading and Working Up Blood Culture Subcultures from Patients with Suspected or Confirmed Viral Hemorrhagic Fever (VHF)

NOTE: identification and antimicrobial susceptibility testing (AST) will NOT be completed from primary subculture plates; subcultures from the primary subcultures plates will be completed and identification and AST will be completed in the “Level 2.5” from these secondary subculture plates taking care to work off of organisms in the third or fourth quadrants away from the inoculation area)

A. Working from Primary Subculture Plates



I. Preparation for Reading and Working Up Blood Culture Subcultures

1. The Designated Senior will:
 - a. initiate and be responsible for completing the [VHF Specimens Handling Log](#) sheet
 - b. gather the [VHF PPE Kit](#) and place it on the bottom shelf of a trolley (**VHF BC Subculture Trolley**)
 - c. gather the necessary laboratory supplies for positive blood cultures working (e.g. LIS labels, loops, subculture agars, QC organisms, CO₂ and anaerobic gas packs) and place them onto the top shelf of the **VHF BC Subculture Trolley**
 - d. assure that the “Level 2.5” laboratory has:
 - i. one large sharps cardboard box lined with two autoclave bags;
 - ii. long cuff nitrile gloves of the right size for the Designated Senior;
 - iii. Virox wipes; and
 - iv. alcohol sanitizer;
 - e. assure that the anteroom has:
 - i. one large sharps cardboard box line with two autoclave bags;
 - ii. glove of the right size for the assistant;
 - iii. Virox wipes, and
 - iv. alcohol sanitizer

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- f. prepare the BSC work area and supplies as follows:
 - i. clean the BSC using Virox wipes; keep the Virox wipes in the BSC
 - ii. lay two blue pads in the clean BSC, one to be used for placing the primary agar subcultures (“dirty blue pad”) and one for placing the secondary subculture plates (“clean blue pad”)
 - iii. place the following onto the “dirty blue pad”
 1. disposable waste container
 - iv. place the following onto the “clean blue pad”
 1. LIS labels
 2. loops
 3. subculture agars
 - g. turn on the Biosafety cabinet (BSC) and check the airflow gauge to ensure that it is running properly
 - h. clear the “Level 2.5” laboratory of any non-essential items
 - i. bring an Autoclave bin on an **Autoclave Trolley** into the “Level 2.5” laboratory anteroom and place by the “Level 2.5” laboratory door
 - j. assign an assistant
2. The assistant will:
 - a. wear standard lab gear
 - b. stay in the anteroom of the “Level 2.5” laboratory waiting to assist the Designated Senior
 - c. move the **Autoclave Trolley** close to the door of the “Level 2.5” laboratory door after the Designated Senior enters the “Level 2.5” laboratory

II. Reading and Working Up Primary Blood Culture Subcultures



1. The Designated Senior will:
 - I. **Don PPE:**
 - a. retrieve the [VHF PPE Kit](#) (from the Spill Kits area) and bring it on the trolley to the hallway outside the “Level 2.5” laboratory anteroom
 - b. put on a standard lab coat

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- c. don PPE using supplies from the [VHF PPE Kit](#) following the order in the [Donning/Doffing VHF PPE appendix](#) with the assistant observing and confirming that donning was completed correctly
 - d. place the [VHF PPE Kit](#) back on the original shelf leaving the lid open to indicate that it is empty
- II. Prepare for Reading and Subculturing Primary Blood Culture Subcultures:**
- a. remove the primary subculture plates snap containers from the incubator and place them on the “dirty blue pad” in the BSC
 - b. wipe the outside of each box with Virox
 - c. replace outer layer of gloves and replace with new gloves
 - d. open the snap container and carefully remove the agar plates and place them onto the “dirty blue pad”
 - e. wipe each agar plate with Virox and let dry
 - f. remove outer layer of gloves and replace with new gloves
 - g. work up all primary subculture plates in the BSC as follows:
 - i. examine primary plates and QC plates
 - ii. leave the agar plate(s) open from which you would like to work
 - iii. remove outer layer of gloves and replace with new gloves
 - iv. prepare subcultures for MALDI and AST as follows:
 1. take care not to touch or work up colonies from the primary inoculum area where blood was deposited (ideally work in the third or fourth quadrant in an area clearly removed from the primary inoculum area)
 2. use one hand to touch the primary agars (designated “dirty hand”) leaving the other hand as the “clean hand” to handle the secondary subculture plates
 3. try to avoid having the “dirty hand” touch the secondary subculture plate; if this is not possible, then use a Virox wipe to handle the secondary subculture plate
 - h. place secondary agar plates into snap incubator containers as follows:
 - i. hand streak the plates
 - ii. allow inoculum to dry on agar plates

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- iii. once dry, close agar plates and wipe the outside of each with a Virox wipe
- iv. carefully place the agar plates and the appropriate QC organism agar plates into NEW snap incubation containers labelled “SECONDARY EBOLA BC SUBCULTURES” taking care not to drop the plates
- v. prepare the CO₂ and anaerobic gas packs
- vi. remove outer layer of gloves and replace with new gloves
- vii. snap close the lids
- viii. wipe the outside of the snap containers with a Virox wipe
- ix. remove outer layer of gloves and replace with new gloves
- x. remove these from the BSC and place them on the laboratory bench by the incubator in the “Level 2.5” Laboratory
- xi. open the incubator and place the snap containers into the incubator carefully
- i. return primary agar plates into the snap containers following the same procedure as above

III. Clean Up

1. Refer to [Clean Up](#) Protocol for Section B. Preparation for Arrival of Specimens from Patients with Suspected or Document VHF (but ignore the reference to **VHF Trolley**) and do NOT remove the “IN USE – DO NOT ENTER” sign on the door to the “Level 2.5” Laboratory until work up of subcultured plates has been completed and blood culture specimens have been removed from the “Level 2.5” laboratory

B. Working From Secondary Subculture Plates

I. Preparation for Reading and Working Up Blood Culture Subcultures



1. The Designated Senior will:
 - a. Follow instructions as above except:
 - i. include preparation materials for MALDI and AST 0.5 McF set up

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II. Reading and Working Up Primary Blood Culture Subcultures

1. The Designated Senior will:
 - a. Follow instructions as above except:
 - i. set up MALDI and AST 0.5 McF in the BSC taking care not to contaminate the MALDI slide or AST 0.5 McF tube
 - ii. place a trolley inside the anteroom (**VHF MALDI/AST trolley**) and place a blue pad and tube rack on its top shelf
 - iii. once MALDI and AST 0.5 McF standards have been set up/completed in the BSC, close tube and wipe with Virox wipes and let dry
 - iv. change outer layer of gloves and replace with new gloves
 - v. transfer separately the prepared MALDI slide and 0.5 McFarland using the “clean hand” out of the BSC, open door, and place the MALDI slide and subculture plate(s) onto the blue pad of the top shelf of the **VHF MALDI/AST Trolley**
2. The assistant will:
 - a. Bring the VHF MALDI/AST Trolley to the main lab for routine processing

III. Clean Up



Refer to [Clean Up](#) Protocol for Section B. Preparation for Arrival of Specimens from Patients with Suspected or Document VHF (but ignore the reference to **VHF Trolley**) and do NOT remove the “IN USE – DO NOT ENTER” sign on the door to the “Level 2.5” Laboratory until work up of subcultured plates has been completed and blood culture specimens have been removed from the “Level 2.5” laboratory

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Microbiology Procedure for Handling Negative Blood Culture Specimens from Patients with Suspected or Confirmed Viral Hemorrhagic Fever (VHF)

A. Removing Negative VHF Blood Culture Bottles from the Incubator

A. The Designated Senior will:



- a. initiate and be responsible for completing the [VHF Specimen Handling Log](#) sheet
- b. wear standard lab gear
- c. obtain a new trolley and prepare it to be the **Negative BC Trolley** and place alcohol hand sanitizer on the top shelf of the trolley
- d. open the designated VHF blood culture drawer
- e. don two pairs of gloves
- f. remove the blood culture bottles from the incubator and place them into blood culture autoclave disposal bag contained within the usual disposal box
- g. remove gloves and place them in the same autoclave disposal bag
- h. perform hand hygiene
- i. close the incubator drawer
- j. using the **Negative BC Trolley**, brings the autoclave disposal bag and box to the autoclave room
- k. close the autoclave box with tape and places it into an autoclave bin and autoclaves it at 121°C for 30 minutes in the gravity cycle
- l. return the **Negative BC Trolley** to the lab for regular use
- m. complete the [VHF Specimens Handling Log](#) sheet and submit it to Occupational Health
- n. when the autoclave is completed, remove the autoclave bin and place the bin and its contents on the floor under the window of the autoclave room
- o. When the VHF diagnosis status of the patient is known:
 - VHF has been ruled out - put the sterilized autoclave bags and Virox wipe into the bin for biological waste disposal (incineration)

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- Patient has been confirmed with VHF – keep the label and discard with instructions from the hospital. Call Support Services through Locating (ext. 5133) to request Support Services Staff to supervise packaging of waste for disposal as per

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Management of Laboratory Exposures and Spills Related to Specimens from Patients with Suspected or Confirmed Viral Hemorrhagic Fever (VHF)

A. Management of Laboratory Exposures

See Management of Laboratory Spills



1. Remove all personnel from the area of the spill
2. The Designated Senior should:
 - a. prepare two sharps cardboard boxes lined with double layer of autoclave bags to be used for waste
 - b. place an autoclave bin on a trolley (**Autoclave Trolley**)
 - c. don water-impermeable gown, fit-tested N95 respirator, face shield, double gloves, foot covers, and hair cover using supplies from the [VHF PPE Kit](#)
 - d. encircle spill with disinfectant solution such as 1:100 dilution of household bleach
 - e. cover spills with absorbent paper towels, liberally covered with disinfectant and left to soak for 30 minutes before being wiped up.
 - f. remove initial material and repeat the process again.
 - g. place all waste sharps box
 - h. tape both autoclave bags inside the sharps box
 - i. tape the sharps box and put it into an autoclave bag
 - j. place the autoclave bagged sharps box in the autoclave bin
 - k. remove all PPE using the recommended order of doffing of PPE as per the posted signage in the level 2.5 laboratory area and put them into the second sharps box
 - l. wash hands
 - m. don gloves
 - n. tie both autoclave bags inside the sharps box
 - o. tape the sharps box and put it into an autoclave bag
 - p. place the autoclave bagged sharps box in the autoclave bin on the **Autoclave trolley**
 - q. wipe gloves

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- r. move the **Autoclave trolley** to the autoclave room
- s. put the autoclave bin into the autoclave and autoclave it at 121°C for 30 minutes at gravity cycle.
- t. removes gloves and washes hands
- u. dons gloves and cleans the **Autoclave trolley** with Virox twice letting it soak and dry between cleans and return the trolley for regular use in the lab
- v. remove gloves and wash hands
- w. return the cleaned **Autoclave trolley** to the lab for regular use
- x. when the autoclave is completed, remove the autoclave bin and place the bin and its contents on the floor under the window of the autoclave room and label all as “Autoclaved EBOLA Waste – Please Do Not Touch”
- y. When the VHF diagnosis status of the patient is known:
 - If VHF has been ruled out - put the sterilized autoclave bags and virox wipe into the bin for biological waste disposal (incineration)
 - If the patient has been confirmed with VHF – keep the label and discard with instructions from the hospital. Call Support Services through Locating (ext. 5133) to request Support Services Staff to supervise packaging of waste for disposal as per
 - Start with small biohazard waste cardboard box, lined with two small red plastic bags
 - Place waste into small red plastic liner, tie off securely
 - Then tie off second red plastic liner securely
 - Then close the small box and tape shut and tape open seams
 - Then place the box into larger red liner
 - The place that package into larger cardboard box and attached the **label UN2814**
- z. call Housekeeping to do a final “VHF Clean” of the spill area
- aa. complete the [VHF Specimen Handling Log](#) sheet and submit it to Occupational Health

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References

ASM Ebola Guidance <https://www.asm.org/images/PSAB/Ebola9-10-14.pdf> (accessed Sept 24, 2014)

PHO Ebola Guidance
<http://www.publichealthontario.ca/en/BrowseByTopic/InfectiousDiseases/Pages/Viral-Hemorrhagic-Fevers.aspx#.VGvldckuAvn>

PHAC Ebola Guidance <http://www.phac-aspc.gc.ca/id-mi/vhf-fvh/ebola-biosafety-biosecure-eng.php> (accessed Sept 24, 2014)

CDC Ebola Guidance <http://www.cdc.gov/vhf/ebola/hcp/index.html> (accessed Sept 24, 2014)

Public Health Agency of Canada website to National Microbiology Laboratory Guide to Services
<https://www.nml-lnm.gc.ca/guide2/labsview.php?labID=23>

Transport of Dangerous Goods Guidelines
[TDG 2014 Compliance Training Reference Manual_176.pdf](#)
[TDG Regulations Section 3 \(relevant sections\) - Shipping Documents \(concise version\).doc](#)

Canadian Biosafety Standards and Guidelines 2013
[Canadian Biosafety Standards and Guidelines 1st edition 2013.pdf](#)

Donning/Doffing Personal Protective Equipment (PPE) References:



- a. WHO PPE Donning/Doffing Guidance
http://who.int/csr/disease/ebola/put_on_ppequipment.pdf?ua=1
http://who.int/csr/disease/ebola/remove_ppequipment.pdf?ua=1
- b. CDC PPE Donning/Doffing Guidance
<http://www.cdc.gov/vhf/ebola/pdf/ppe-poster.pdf>

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- c. University of Nebraska PPE Donning/Doffing Guidance
http://app1.unmc.edu/nursing/heroes/ppe_posters_vhf.cfm



Waste Disposal

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Appendices

A. Ordering Information for Transportation of Dangerous Goods Class 6.2 Placards



1. Order Non-Worded Class 6.2 Infectious Substance Placard
 - Specify under Comment Section that UN2814 is to be printed in centre of placard
 - Online store: http://www.thecompliancecenter.com/placards/non_worded/class6/class62.htm

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**B. Donning/Doffing Viral Hemorrhagic Fever (VHF) Personal Protective Equipment (PPE)
SUSPECT VHF PRECAUTIONS FOR STAFF DONNING CHECKLIST:**

****Check off each item after completion:**



	Donning must be done in the presence of a <u>trained observer</u>
Donning PPE for VHF	
	1. Remove all unnecessary layers of clothing.
	2. Ensure all hand and wrist jewelry is removed and hair tied back.
	3. Put on shoe covers and fold down the top to create a lip to facilitate doffing.
	4. Perform hand hygiene.
	5. Put on “fit tested” N95 respirator. Pinch nose piece. Perform seal check.
	6. Put on white impermeable hood. Ensure straps are through the loops on the sides. Tie the straps at back of head. Lay the edges of the hood flat against body.
	7. Put on long cuff gloves.
	8. Put on water resistant gown:
	a. Reach inside the front of the gown to check that edges of white hood are still flat against body.
	b. Secure neck Velcro of gown.
	c. DO NOT TIE UP THE INSIDE OF THE GOWN. Use tape instead to secure the gown on the inside.
	d. Tie up the side of gown on the outside.
	e. Use tape:
	i. to secure the gown from opening on the back.
	ii. to assure the cuff of the gown stays over first pair of gloves.
	9. Put on face shield. Ensure that 0.5 cm of hood can be seen below head band.
	10. Put on plastic cap. Ensure that it covers foam band of face shield. (not for labs)
	11. Put on a second pair of long cuff gloves over the cuff of gown.

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****Have the trained observer check you once donning is complete to make sure you have done it right.**

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SUSPECT VHF PRECAUTION FOR STAFF DOFFING CHECKLIST:

***** KEEP HANDS BELOW SHOULDERS AT ALL TIMES*****

****Check off each item after completion:**



	<u>Doffing must be done in the presence of a trained observer</u>
	Doffing PPE for VHF on DIRTY SIDE OF ANTEROOM
	1. Remove outer gloves , and dispose into the biohazard waste bin.
	2. Untie side of gown.
	3. Remove gown and inner gloves together by crossing arms and pulling gown from upper arm area and rolling gown inside out being sure that gloves are removed with gown. Dispose of gown and gloves into biohazard waste bin.
	4. Perform hand hygiene.
	5. <i>Put on new gloves from clean side without stepping onto clean side.</i>
	6. Leaning forward and facing the biohazard waste bin on dirty side:
	i. Remove cap into biohazard waste bin. (not for labs)
	ii. Remove face shield (lift up and away from body) into biohazard waste bin.
	iii. Remove hood (Untie from the back. Pinch bottom of hood from the front with one hand. With the other hand, lift the top up and pull it up and away). Dispose hood into biohazard waste bin
	7. Remove gloves into the biohazard waste bin.
	8. Perform hand hygiene.
	9. <i>Put on new regular <u>face shield</u> and <u>new gloves</u> from clean side without stepping onto clean side.</i>
	10. With feet on the dirty side, sit down on chair close to the clean side and remove shoe covers as follows:
	i. Start with leg closer to clean side.
	ii. Use the lip created during donning to pull the shoe cover inside out down to heel and over the foot being careful not to touch own clothing. After doffing shoe cover, place foot onto clean side.
	iii. Discard shoe cover into the biohazard waste bin.

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	iv. Repeat with other leg
Doffing PPE for EBV on CLEAN SIDE OF ANTEROOM	
	11. Remove gloves into the biohazard waste bin.
	12. Perform hand hygiene.
	13. Remove face shield (lift up and away) into the biohazard waste bin.
	14. Remove N95 respirator (bottom strap first, then top strap) into the biohazard waste bin.
	15. Perform hand hygiene.

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C. VHF Specimens Handling Log



Date	Start Time	End Time	Patient Name	Staff Name	Staff Signature	Task

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D. Kit Contents

vi. [VHF PPE Kit](#)

Inside PPE bag:

- 1- SmartGown Impervious x-long
- 1- Bonnet cap (not for labs)
- 1- IsoClean Hood
- 1- Face shield with Protective Drape
- 1- Pair of Impervious HighTop boot covers

Fit-tested N95 respirator
 Nitrile gloves, long cuff (green)
 Duct tape
 Opaque tape

vii. [VHF Specimen Kit](#)

Virox wipes
 Absorbent blue pads
 Specimen Transport Bags
 Discard container
 2 – face shields for doffing



viii. [VHF Blood Culture Kit](#)

Virox wipes
 Absorbent blue pads
 Loops
 Glass Slides

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Plastic slide transport containers for Methanol and Formalin fixatives
 Anaerobic/CO₂ Boxes
 Anaerobic gas packs
 CO₂ gas packs
 Specimen Transport Bags
 Sharps container
 2 – face shields for doffing PPE

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E. Viral Hemorrhagic Fever (VHF) Epidemiologic Tool



Request that the specimen be tested: <input type="checkbox"/> During daytime lab hours <input type="checkbox"/> Immediately	LIS LABELS: <div style="border: 1px solid black; border-radius: 10px; width: 200px; height: 60px; margin-top: 5px;"></div>
Name: _____	
Date of birth: ____/____/____ (yyyy/mm/dd) Gender: _____	
MRN: _____ Health Card Number: _____	
City and province of sample collection: _____	
Date of onset of first symptom(s): ____/____/____ (yyyy/mm/dd)	
Name of the city/ies (and country/ies if outside Canada) where the individual had high risk exposures: _____	
Epidemiologic risk factors within 21 days before the onset of symptoms: <input type="checkbox"/> residence in or travel to an area where VHF transmission is active <input type="checkbox"/> healthcare workers/personnel ² who have spent time in a setting where VHF patients are being assessed or cared for in an VHF-affected area <input type="checkbox"/> other patients and visitors who spent time in a healthcare facility where VHF patients are being treated <input type="checkbox"/> household members of a VHF patient <input type="checkbox"/> percutaneous or mucous membrane exposure or direct skin contact with body fluids of a person with a confirmed or probable case of VHF	

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- laboratory processing of body fluids of probable or confirmed VHF cases
- participation in funeral rites or other direct exposure to human remains in the geographic area where the outbreak is occurring
- persons who had direct unprotected contact with animal reservoir or arthropod vector from VHF-affected country

Manifestations:

- | | | |
|--|--|---|
| <input type="checkbox"/> Fever ($\geq 38.6^{\circ}\text{C}$) | <input type="checkbox"/> Difficulty swallowing | <input type="checkbox"/> Unexplained bleeding |
| <input type="checkbox"/> Stomach Pain | <input type="checkbox"/> Sore throat | <input type="checkbox"/> Neurological symptoms (e.g. seizure, coma) |
| <input type="checkbox"/> Hematemesis | <input type="checkbox"/> Difficulty breathing | <input type="checkbox"/> Headaches |
| <input type="checkbox"/> Vomiting | <input type="checkbox"/> Intense coughing | <input type="checkbox"/> Lethargy |
| <input type="checkbox"/> Diarrhoea | <input type="checkbox"/> Red eyes | <input type="checkbox"/> Myalgia |
| <input type="checkbox"/> Bloody stools | | <input type="checkbox"/> Skin rash |
| <input type="checkbox"/> Anorexia | | |

¹Based on August 20, 2014 NML ERAP Epidemiologic Tool

²Healthcare workers: individuals who provide health care or support services such as nurses, nurse practitioners, physicians, dentists, paramedics, some emergency first responders, allied health professionals, unregulated healthcare providers, clinical instructors and students, volunteers and housekeeping staff; have varying degree of responsibility related to the health care they provide, depending on their level of education and their specific job/responsibilities

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Infection Control Fan Out List



Please see Paradigm Document Viral Hemorrhagic Fever Infection Control Fanout List QFSMI03002a

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Record of Edited Revisions

Page Number / Item	Date of Revision	Signature of Approval
Procedure expanded	September 25, 2014	Dr. T. Mazzulli
Modified donning and doffing procedure for new PPE supplies	October 18, 2014	Dr. T. Mazzulli
Changed VHF PCR send out to PHOL and NML	October 18, 2014	Dr. T. Mazzulli
Added Link to Stericycle waste disposal instructions	October 18, 2014	Dr. T. Mazzulli
Added link to Micro staff training list	October 18, 2014	Dr. T. Mazzulli
Added Assessing risk of EVD in ED patients	October 18, 2014	Dr. T. Mazzulli
Remove send out to NML; updated PHOL delivery procedure	December 30, 2014	Dr. T. Mazzulli
Changed specimen tubes requirement for VHF detection	December 30, 2014	Dr. T. Mazzulli
Added tab for process if patient arrives from a clinic	December 30, 2014	Dr. T. Mazzulli
Updated donning and doffing procedure	December 30, 2014	Dr. T. Mazzulli
Revised procedure; Updated transport procedure: -Removed NML and PHL send out/shipping requirements -Removed labels and forms for shipping reference link section	August 18, 2015	Dr. T. Mazzulli
In TOC added links under notification and approval of VHF testing : 1. Assessing risk of EVD in ED patients, 2. Clinic outpatient ebola notification overview and 3. ED ebola notification overview. <i>Section: Notification and Approval of VHF Testing</i> Included documents links to the ED communications flowchart and outpatient area communications flowchart	September 1, 2015	Dr. T. Mazzulli
Annual Review	July 31, 2016	Dr. T. Mazzulli
Removed VHF IC Fanout List to own Document	October 2, 2017	Dr. T. Mazzulli

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