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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 by infection control at any site:
   a. the infection control physician will arrange for a phone call or electronic meeting between:
      i. the most responsible physician
      ii. the microbiologist-on-call
      iii. the infection control physician
to discuss the patient’s clinical and epidemiologic information and whether VHF testing is deemed warranted using an established Viral Hemorrhagic Fever (VHF) Epidemiologic Tool and Appendix I of the PHO Interim IPAC Recommendations for the Care of Individuals with Suspect or Confirmed EVD as guidance
   b. NOTE: 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 infection control physician will arrange for Toronto Public Health and the patient’s resident address local public health unit (Reportable Diseases to the Medical Office Health QPCMI16001) to be notified of the suspect case
   b. the microbiologist-on-call will:
3. If approval for VHF testing is obtained:

   a. the microbiologist-on-call will:

      i. immediately notify the Infection Control Physician who will notify the Most Responsible Physician and hospital administration fan-out list

      ii. notify the UHN/MSH Department of Microbiology by emailing all microbiologists (tony.mazzulli@sinaihealth.ca; susan.poutanen@sinaihealth.ca, and vanessa.allen@sinaihealth.ca) and laboratory seniors, charges, and supervisor via microbiologyspecialqueries@sinaihealth.ca.

      iii. notify the Microbiology Designated Senior by phone by calling 416-586-4800 x 2610 or x5562 of the anticipated specimens:

         For Sinai Health patients, Microbiology should receive:

         - 2 blood culture sets (to be processed at UHN/MSH Department of Microbiology)
         - 2 EDTA for VHF detection (to be processed at PHOL/NML)
         - 1 EDTA for malaria detection (to be processed at PHOL)
         - 1 EDTA tube for CBC (to be processed at UHN)
         - 1 sodium citrate tube for INR (to be processed at UHN)
• 1 heparin tube for electrolytes, creatinine, urea, glucose, AST, ALT, ALP, bilirubin, albumin, protein, calcium, troponin (to be processed at UHN)

For University Health Network patients, Microbiology should receive:
• 2 blood culture sets (to be processed at UHN/MSH Department of Microbiology)

iv. notify the relevant core laboratory personnel by calling:

For Mount Sinai Hospital specimens:
• contact the rapid response lab manager, Kathy Yates (x4499 Mobile: 437-223-8318) and notify them of the blood work being sent out by Microbiology on their behalf and of the expected upcoming results from UHN core laboratory

For University Health Network (UHN) specimens:
1. contact the UHN LMP Manager on-call via UHN locating
   a. notify them of the upcoming specimens if they have not yet been made aware from the hospital administration fan-out list notification
   b. provide the ERAP number (expected to be ERP2-0746) for all specimen transportation
   c. confirm how to activate and deactivate the ERAP based on instructions from PHOL
2. if requested, provide back up Class 6.2 Infectious Substance Placards available in the microbiology laboratory in the VHF Specimen Kit
   a. discuss the need for Category A Transportation and use KJV courier if needed.
      i. Refer to Send out SOP for instructions (page 30)
   b. Refer to internal manual “QIMMII06006” for login information

v. for specimens arriving from sites other than Mount Sinai Hospital and University Health Network sites:

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• contact the site’s locating and ask to speak to the laboratory director to:
  a. discuss the need for Category A Transportation and use KJV courier if needed.
  • Refer to Send out SOP for instructions (page 30)
  • Refer to internal manual “QIMMII06006” for login information
  b. provide the ERAP number (expected to be ERP2-0746)
  c. confirm how to activate and de-active the ERAP based on instructions from PHOL
B. Preparation for Arrival of Specimens from Patients with Suspected or Documented VHF

1. The Designated Senior Technologist will:
   a. initiate and be responsible for completing the VHF Specimens Handling Log sheet
   b. assign an assistant to work with the Designated Senior.

2. 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.

3. The Designated Senior will:

   For Mount Sinai Hospital specimens:
   I. Print VHF Specimens Handling Log and be responsible for ensuring the log is filled by all staff handling the specimens of the case

   II. Prepare Labels, Requisitions and Shipping Items:
      a. contact KJV courier to inform them of the upcoming specimens going to PHOL
         • Refer to Send out SOP for instructions (page 30)
         • Refer to internal manual “QIMMII06006” for login information

         Note: Hospital support services supervisor (x2026) will transport the specimens to the lab by foot and will wait upon drop off for Biochemistry and Hematology samples going to UHN Core Lab to be received so that they can transport them by foot through the hospital tunnel system without the need to activate ERAP.

      b. receive and print specimen labels and requisitions for all the anticipated specimens below in the LIS, i.e.
         i. 2 blood culture sets (4 bottles) (to be processed at UHN/MSH Department of Microbiology)
ii. **3 EDTA tubes: 2 for VHF detection and 1 for malaria detection** (to be processed at PHOL/NML) – print a PHOL requisition from the LIS.

---

**Ebola Test request to PHOL** – when ordering VHF detection, different assays exist depending on the ebolavirus strain. Consult with Microbiologist on orderable for current request.

*For the 2022 Ebola epidemic, please order on PHOL requisition:*
- Sudan ebolavirus detection,
- Crimean-Congo hemorrhagic fever virus detection and
- Rift Valley fever virus detection

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iii. **1 EDTA tube for CBC* & 1 sodium citrate tube for PT/INR**

iv. **1 heparin tube for electrolytes, creatinine, urea, glucose, AST, ALT, ALP, bilirubin, albumin, protein, calcium, troponin I***

*These are all to be processed at UHN Core Lab – complete a UHN requisition located in the VHF kit or in the internal manual “VHF UHN Biochemistry & Hematology Test Requisition”.

Photocopy UHN requisition once LIS labels are applied and send a copy by pneumatic tube to 11th floor PLM.

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c. Complete and sign [Shippers-Declaration MSH to PHL](#) (3 original copies needed) for specimens for VHF and malaria testing at PHOL following **Class 6.2 Category A (UN2814) “P620 / type 1A” shipping procedures.**

Ensure KJV waybill is printed for shipping.

Keep one copy of shippers declaration and waybill for filing at the Seniors Desk.

*Not required for internal UHN samples*
d. Prepare two empty Category A shipping boxes as per **TDG procedures.**
   i. One (1) with ERAP notification for PHOL samples. An icepack is required for shipping to PHOL.
   ii. Two (2) without ERAP for UHN samples.

*For University Health Network specimens:*

I. **Print VHF Specimens Handling Log:**
   a. print [VHF Specimens Handling Log](#) and be responsible for ensuring the log is filled by all staff handling the specimens of the case

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

*For Mount Sinai Hospital and University Health Network specimens:*

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 lab hallways door to the anteroom as well as the Level 2.5 laboratory door. **Note:** No persons will have access to the mycology department while a VHF samples is being processed until housekeeping has completed a terminal clean.
   c. assure that the “Level 2.5” laboratory has:
      i. one garbage lined with two autoclave bags;
      ii. long cuff nitrile gloves of the right size for the Designated Senior;
      iii. Accel/Virox wipes container
      iv. Liquid Accel/Virox
      v. alcohol sanitizer
      vi. shipping tape
      vii. parafilm
      viii. 2 face shields on the clean side of the bench

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d. assure that the anteroom has:
   i. VHF waste drug (delivered automatically by support services (x2026) once hospital VHF protocol is initiated.
   ii. glove of the right size for the assistant;
   iii. Virox wipes container
   iv. Alcohol sanitizer
   v. stool
e. clear the “Level 2.5” laboratory and anteroom of any non-essential items
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:
   iv. clean the BSC using Virox wipes; keep the Virox wipes in the BSC
   v. 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”)
   vi. place the following onto the “dirty blue pad”:
      1. disposable waste container
   vii. place the following onto the “clean blue pad”:
       1. LIS labels
       2. absorbent transport sleeve
       3. clean plastic specimen bags,
   viii. place 10 pairs of gloves beside the BSC on the counter

IV. Prepare the Anteroom of the “Level 2.5” Laboratory
a. obtain a new trolley and prepare it to be the UHN/PHOL transport trolley as follows:
   i. place the following items on the top shelf of the trolley
      1. opened pre-labelled UHN hard plastic shipping container (for MSH samples only)
      2. opened pre-labelled PHOL hard plastic shipping container
   ii. place the following items on a lower shelf of the trolley

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1. pre-labelled UHN cardboard shipping box (for MSH samples only)
2. pre-labelled PHOL cardboard shipping box
3. complete shipping paperwork
   iii. place the trolley in the anteroom outside of the “Level 2.5” laboratory to keep these items clean
b. 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

V. **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

VI. **Return VHF Trolley and Kits and Wait for Specimens:**
   a. wheel the VHF trolley with the empty VHF Specimen Kit container back to the receiving area
      i. put the empty VHF PPE Kit and VHF Specimen Kit containers back onto the shelf left open to indicate that it is empty
      ii. keep the VHF trolley in the receiving area ready to receive the VHF specimens
   b. wait for specimen to arrive

4. The assistant will:
   a. wear standard lab gear with double gloves

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C. Receiving Specimens from Patients with Suspected or Documented VHF

1. When the specimens arrive in the laboratory, specimen management should NOT receive or open any of the containers. Direct the porter (support services supervisor) to the garbed Designated Senior.

2. For Mount Sinai Hospital specimens:
   a. specimens will be packaged inside a clean hard plastic specimen container that will be packaged inside a hard shelled courier transport tote and delivered to Microbiology by a porter. The Translogic system will NOT be used. The porter will wait for UHN samples to be ready to deliver them to UHN.

3. For University Health Network (UHN) specimens:
   a. 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.

4. The Designated Senior will:
   a. receive the specimens on the VHF trolley
   b. carefully bring the VHF trolley into the “Level 2.5” laboratory.

5. The assistant will:
   a. facilitate transport by opening doors or the like for the Designated Senior as needed
   b. bring the UHN/PHOL transport trolley close to the door after the Designated Senior has entered into the “Level 2.5” laboratory
   c. stay in the anteroom of the “Level 2.5” laboratory waiting to assist the Designated Senior.

D. Preparation of Specimens for VHF Testing at UHN/PHOL in the “Level 2.5” Laboratory

1. The Designated Senior will:
   a. open the courier bag on the VHF trolley and place the hard plastic specimen transport containers inside the BSC onto the “dirty blue pad”
   b. wipe down the inside of the courier bag with Virox wipes and place the courier bag onto the bottom of the trolley
   c. open all the hard plastic specimen containers inside the BSC
d. remove the biosafety bags one at a time and place the bags with specimens on the “dirty blue pad”

e. one by one, remove the specimen tubes from their biosafety bag and wipe the tubes including the tops with Virox wipes and place them on the “clean blue pad”

f. remove outer layer of gloves and replace with new ones

g. attach LIS labels on the tubes as follows:

i. **2 EDTA for VHF viral detection** (to be processed at PHOL)

ii. **EDTA for malaria detection** (to be processed at PHOL)

iii. **EDTA for CBC and Blue top for PT/INR** (to be processed at UHN)

iv. **Green top for Biochemistry** (to be processed at UHN)

h. Apply parafilm to all the tubes

i. Place each of the 4 groups of tubes into to absorbent transport sleeve and place them into a new plastic biosafety specimen bag and seal the bag according to their grouping above.

j. wipe the outside of the biosafety specimen bags with a Virox wipe

k. remove outer layer of gloves and replace with new ones

l. open the door and:

i. place the **biosafety bag containing** specimen tubes into the appropriate PHOL and UHN hard plastic transport container being careful not to touch the outside of the container.

E. Preparation of PHOL Shipment in the “Level 2.5” Laboratory Anteroom

1. The assistant will:

a. close PHOL hard plastic transport container

b. wipe the outside of the container with a Virox wipe

c. remove the outer layer of gloves

d. place the container inside the pre-labelled PHOL cardboard shipping with requisition between container and shipping box.

e. remove gloves and perform hand hygiene

f. close the cardboard shipping box and place the appropriate shipping documents into a zip lock bag, tape the zip lock bag on one side to the top of the box preparing it for shipping.
g. bring the **UHN/PHOL transport trolley** to the seniors bench.
   i. Provide UHN boxes to the porter (support services supervisor) to deliver to UHN 3EB-347 via underground tunnel.
   Directions: The TGH Core Laboratory is located on the 3rd floor of the East (aka Eaton) Wing, room 347. Take the East Wing (aka Eaton) elevators to the 3rd floor and follow the signs to ‘Specimen Delivery’
   ii. Assign a technologist to wait with it until the shipment is picked up by KJV; once picked up, the **PHOL transport trolley** can be returned to the lab for regular use

h. return to the anteroom
   i. bring the anteroom **BC transport trolley** close to the door

F. **Preparation of Blood Cultures in the “Level 2.5” Laboratory**
   1. The Designated Senior will:
      a. return to the 2 blood culture sets in the biosafety cabinet
      b. remove the blood culture bottles from the specimen container and place them on the “dirty blue pad”
      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**

G. **Incubating Blood Cultures**
   1. The assistant will:
      a. don two pairs of gloves

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b. close the hard plastic shipping container
c. remove gloves
d. perform hand hygiene
e. transport the **BC transport trolley** to the blood culture incubator
f. once at the blood culture incubator, load the bottle in the designated VHF blood culture Virtuo unit **manually**, (Do not load through conveyor belt)
g. remove the blood culture bottles from the hard plastic shipping container and biosafety bag using the trolley as a workstation
h. Have a designate help to touch screens or open/close units. Place the blood culture bottles in the designated VHF slots in the Virtuo taking care not to touch anything but the blood culture bottles
i. remove gloves and places them on the blue pad
j. perform hand hygiene
k. closes the incubator drawer
l. brings the **BC transport trolley** back into the anteroom of the “Level 2.5” laboratory
m. don two pairs of gloves
n. remove the blue pad and specimen bag and gloves from the top of the trolley and place them in the double lined garbage.
o. remove the outer layer of gloves ad replace with new ones
p. wipe down the **BC transport trolley** with liquid Virox and Virox wipes two times keeping it wet for 5 minutes each time
q. removes gloves
r. perform hand hygiene
s. Push waste drum close the door. Move anteroom double bagged garbage close to door
H. **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 double bagged biohazard garbage.
   
   b. place unused gloves inside the double bagged biohazard garbage
   
   c. remove outer layer of gloves and replace with new gloves
   
   d. 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)
   
   e. tie both layers of the biohazard bag
   
   f. open the door
   
   g. place the closed garbage bag in the waste drum.
   
   h. bring the anteroom double bagged garbage into the “Level 2.5” laboratory

2. The assistant will: return to anteroom.

3. The Designated Senior will:

   **I. Doff PPE:**
   
   a. wait for the assistant to return
   
   b. 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. When moving into the anteroom with the garbage, the assistant must move to the hallway.

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c. put all doffed PPE into the double bagged biohazard garbage  
d. perform hand hygiene  
e. tape both layers of double biohazard garbage  
f. wipe the Virox wipe container with a Virox wipe and use the Virox wipe to open the door  
g. place garbage into the waste drum.  

II. Return VHF Trolley:  
a. remove the cleaned **VHF trolley** from the “Level 2.5” laboratory and return the trolley to regular use  

III. Restocking Supplies, Final Housekeeping Clean, Specimen Handling Log  
a. Ask the designated safety officer asking him/her to:  
   1. restock VHF courier bag and return it to the sender  
   2. restock the lab’s **VHF PPE kit** and **VHF Specimen Kit**  
b. call support services to secure and remove drum. ext. 5133  
c. provide support services a new VHF kit to deliver to ward.  
d. call Housekeeping to do a final “VHF Clean” in the “Level 2.5” laboratory  
e. once cleaned by Housekeeping, remove the “DO NOT ENTER-Ebola Specimen In Progress” sign on the door to the “Level 2.5” and hallway Laboratory door.  
f. complete the **VHF Specimens Handling Log** sheet  

Microbiology Procedure for Handling and Processing Positive Blood Culture Specimens from Patients with Suspected or 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  

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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 garbed lined with two biohazard bags;
      ii. long cuff nitrile gloves of the right size for the Designated Senior;
      iii. Virox wipes; and
      iv. alcohol sanitizer;
   f. assure that the anteroom has:
      i. one large garbed lined with two biohazard bags;
      ii. glove of the right size for the assistant;
      iii. Virox wipes, and
      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 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% 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

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:
   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
   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-biosecurite-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

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)
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

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iv. alcohol sanitizer

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. 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

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
      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
      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

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
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)
- 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 Stericycle EVD Waste Disposal Instructions.pdf
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 MSH Monitoring and Management of Personnel potentially exposed to Ebola Virus (EVD)

B. 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
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 routine 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 Stericycle EVD Waste Disposal Instructions.pdf
      o Start with small biohazard waste cardboard box, lined with two small red plastic bags
      o Place waste into small red plastic liner, tie off securely
      o Then tie off second red plastic liner securely
      o Then close the small box and tape shut and tape open seams
      o Then place the box into larger red liner
      o 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
References

PHO VHF Testing Information

PHO Laboratory Guidance on VHF Testing Recommendation

PHO Laboratory Guidance on ERAP

PHO Technical Brief on Interim VHF IPAC Recommendations

Ministry of Health Public Health Management of VHF Interim Guidance


CDC VHF PPE Donning/Doffing Guidance
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
B. Donning/Doffing Viral Hemorrhagic Fever (VHF) Personal Protective Equipment (PPE)

SUSPECT VHF PRECAUTIONS FOR STAFF DONNING CHECKLIST:

**Check off each item after completion:

<table>
<thead>
<tr>
<th>Donning must be done in the presence of a “buddy”</th>
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**DONNING PPE for VHF**

1. Remove all unnecessary layers of clothing, landyard.
2. Ensure all hand and wrist jewelry is removed and hair tied back.
3. Review contents of PPE Kit.
4. Put on shoe covers and fold down the top to create a lip to facilitate doffing.
5. Perform hand hygiene.
6. Put on “fit tested” N95 respirator. *(Not in kit)*
   - Pinch nose piece. Perform seal check.
7. Put on white impermeable hood.
   - a. Pull hood down stretching front opening from above your brown and tuck bottom edge of elastic under your chin.
   - b. Lay the edges of the hood flat against body (front and back)
8. Put on water resistant gown.
   - It is intentionally long and should extend below the top of shoe covers
   - 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 edge of the gown.
   - d. Tie up the side of gown on the outside.
   - e. Have you buddy check gown is secure, hood/edges tucked and no exposed areas of clothing.
10. Put on face shield.
   a. Extend flap at bottom of face shield before you put it on.
   b. Have buddy ensure that 0.5 cm of hood can be seen below foam head band and pinch tab at bottom of flap

11. Put on plastic cap **(not required for laboratories staff)**. Ensure that it covers foam band of face shield.

12. Put on first pair of long cuff gloves over the cuff of gown. Tape cuff of gloves to front and back of sleeves (to ensure gloves come off together with gown when doffing).

13. Put on second pair of long cuff gloves over the cuff of gown.

**Have your buddy check you once donning is complete to make sure you have done it right.**
**SUSPECT VHF PRECAUTIONS FOR STAFF DOFFING CHECKLIST:**

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

**Check off each item after completion:**

<table>
<thead>
<tr>
<th><strong>Doffing must be done in the presence of a “buddy”</strong></th>
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**DOFFING PPE for VHF on DIRTY SIDE OF ANTEROOM**

1. Remove exterior gloves, and dispose into the biohazard waste bin *(lab staff should remove and dispose these gloves inside Biosafety cabinet)*
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. **Put on new gloves from clean side without stepping onto clean side.**
6. Leaning forward and facing the biohazard waste bin on dirty side:
   i. Remove cap into biohazard waste bin *(not required for laboratories staff)*.
   ii. Remove face shield (from behind 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. **Put on new regular face shield and new gloves from clean side without stepping onto clean side.**
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.
iv. Repeat with other leg

DOFFING PPE for VHF on CLEAN SIDE OF ANTEROOM

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<tr>
<td>11.</td>
<td>Remove gloves into the biohazard waste bin.</td>
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<td>12.</td>
<td>Perform hand hygiene.</td>
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<td>13.</td>
<td>Remove face shield (lift up and away) into the biohazard waste bin.</td>
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<td>14.</td>
<td>Remove N95 respirator (bottom strap first, then top strap) into the biohazard waste bin.</td>
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<td>15.</td>
<td>Perform hand hygiene.</td>
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C. Forms, Requisitions and Labels

1. Public Health Agency of Canada – Requisition for Special Pathogens at NML
   NML Requisition-form-Special Pathogens

2. Shipping Declaration forms:
   Shippers-Declaration MSH to PHL

3. Labels:
   Mount Sinai Hospital - Tony Mazzulli.doc
### D. VHF Specimens Handling Log

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<th>Date</th>
<th>Start Time</th>
<th>End Time</th>
<th>Patient Name</th>
<th>Staff Name</th>
<th>Staff Signature</th>
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E. Kit Contents

vi. **VHF PPE Kit**

   Inside PPE bag:
   - 1- SmartGown Impervious x-long
   - 1- Bonnet cap
   - 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
   TDG transport containers for NML and PHL
   Absorbent transport sleeves
   Specimen Transport Bags
   TDG Declaration Form (filled)
   Shipping requirement checklist
   Discard container
   2 – face shields for doffing

viii. **VHF Blood Culture Kit**

   Virox wipes
   Absorbent blue pads
   Loops
   Glass Slides
   Plastic slide transport containers for Methanol and Formalin fixatives
   Anaerobic/CO\textsubscript{2} Boxes
   Anaerobic gas packs
   CO\textsubscript{2} gas packs
   Specimen Transport Bags
   Sharps container
   2 – face shields for doffing PPE
Instructions for filling forms:

Requisition for Special Pathogens - Instructions 2.pdf

Shipping Label Instructions for Manual-MSH to PHL.pdf

Shipping Box lid - packing instructions for Manual.pdf

Shipping Box-Infectious Substance Instructions for Manual.pdf
I. Microbiology VHF Trained Staff List:

VHF Training Schedule
## Record of Edited Revisions

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<th>Signature of Approval</th>
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<tr>
<td>Annual Review</td>
<td>October 02, 2017</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Minor formatting changes</td>
<td>August 13, 2018</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Annual Review</td>
<td>October 15, 2018</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Annual Review</td>
<td>December 21, 2019</td>
<td>Dr. T. Mazzulli</td>
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Full document review included in all updates. Biennial review conducted when no revision had been made within 2 years.

| Minor formatting change            | April 09, 2021     | Jessica Bourke         |
| Updated full document              | February 24, 2023  | Jessica Bourke         |