Viral Haemorrhagic Fevers
Laboratory Guidance for Samples Handling and Management

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**Aim:**
To provide a set of policies and procedures for handling and testing of samples from patients with suspected Acute viral haemorrhagic fevers (VHFs).

**Introduction:**

Acute viral haemorrhagic fever syndromes (VHFs) can be caused by many viruses including: dengue, Ebola-Marburg viral diseases, Lassa fever, Yellow fever, Rift Valley fever, Hantavirus infections, Crimean–Congo Hemorrhagic fever (CCHF), West Nile Fever and other viruses. VHFs are not indigenous to Oman. Sporadic cases of CCHF due to imported infected sheep and goats from neighboring countries have led to local transmission. Several cases of CCHF have been reported since the first case detected in 1995. All dengue fever cases to date are acquired outside Oman.

VHF may be suspected in a wide range of situations varying from asymptomatic carriers through all phases of the illness, or even a retrospective diagnosis after the patient has recovered or died. Initial clinical presentation may be non-specific including fever, pharyngitis, and myalgia with or without haemorrhagic manifestations. As a result, recognition of cases may be slow and have been cared for in other institutions before being transferred to a tertiary care facility.

All cases of VHFs whether single or cluster, should be notified early. Heightened surveillance by the Ministry of Health is aimed at early recognition of cases in order to prevent epidemics and outbreaks.
General instructions:

A) Sample Collection Instructions to Clinicians:

1. The laboratory should be notified in advance that samples from a case of SUSPECTED VHF are being sent. This must be done by a senior clinical staff.

2. A Senior staff/consultant should be informed and should liaise with attending clinicians on an agreed set of investigations.

3. ONLY ESSENTIAL INVESTIGATIONS SHOULD BE SENT TO THE LABORATORY UNTIL THE DIAGNOSIS IS VERIFIED AND VHF IS EXCLUDED.

4. Personal protective equipments (PPEs) described for hospital staff should be worn when collecting laboratory specimens (please refer to infection control guidelines). Double gloving is recommended to facilitate the decontamination of the exterior of the specimen container.

5. The entire outside surface of each specimen container should be wiped with a disinfectant solution (such as 1:100 dilution of household bleach- hypochlorite) and a label should be attached before specimen collection (if label will withstand cleaning with disinfectant) bearing the patient's name, hospital number, source of the specimen, date of collection, and the nature of the suspected infection. Then the outer layer of gloves can be removed.

6. Clinical laboratory specimens should each be wrapped with absorbent material such as tissue and placed into double sealable plastic bags and then sealed.

7. The laboratory request form for each sample should be placed in a separate pocket of the plastic bag (NOT inside the sample compartment).

8. The outside of these bags should be wiped with a disinfectant solution such as 1:100 dilution of household bleach (hypochlorite) before leaving the patient's room.

9. Samples can then be transported in durable, leak proof containers directly to the specimen handling area of the laboratory. Samples should not be left unattended while awaiting transportation.

10. Laboratory staff should be alerted to the nature of the specimens, which should remain in the custody of a designated person until testing is done.

11. Specimens should be transported in person and handed directly to a senior staff member in the designated receiving area (DRA) – NEVER through the pneumatic tube system (where available).

See blood collection guide (Appendix II and IX)
B) General Instructions to all Laboratory Staff:

1. Precautions to be used must be based on enhanced Biosafety level 2 (BSL2) environment.

2. Appropriate risk assessments should be in place and evaluate the risks of each analytical procedure and the appropriate control measures needed.

3. Wherever possible, samples must be inactivated before they are tested, in a BSL2 cabinet using enhanced standard and droplet precautions (See Appendix I and II).

4. Personnel testing samples should be kept to a minimum and include only senior staffs – preferably one per laboratory section and after other staff have vacated the area, following the protocol set out in the Health & Safety (HSE) manual.

5. After testing, remaining samples should be returned to this area (DRA) for safe disposal or storage.

6. Specimen handling and storage should be kept to a minimum

7. If specimens are not inactivated:
   - Specimens must be processed in a BSL2 cabinet (in DRA)
   - Specimens should be processed in a segregated area (DRA) using a dedicated blood/gas analyzer or similar standalone machine. Protocols will need to be in place for safe processing, handling and disposal including waste from the analyzer.
   - Consideration should be given to using face protection and fit tested N95 mask for practices and procedures that have been assessed as likely to create splashes or aerosolisation.
   (see Appendix II)

8. Routine cleaning and disinfecting procedures can be used for automated analyzers; analyzers should be disinfected after use as recommended by the manufacturer or with a 500 parts per million solutions (1:100 dilution) of sodium hypochlorite (1/4 cup of household bleach to 1 gallon water).

9. After use, the work surfaces should be disinfected with 1,000ppm chlorine.

10. In case of accidental spills, please follow the instructions in Appendix III

11. In case a staff accidentally gets exposed to a potentially infectious material, please follow the instructions in Appendix IV. A form must be filled for such incidents (see Appendix VIII)
The Designated Receiving Area (DRA):

The following are considered the minimal requirement of the DRA:

- The room should be physically separated from other areas and can be easily sealed for decontamination.

- The room should be equipped with BSC class II, sink, water bath for samples inactivation, and centrifuge. Refrigerator and -20°C freezer are required if samples need to be stored, storage outside this area should be avoided.

- The door of this area should be closed at all the time while working with VHF samples and have a sign (Strict access. DO NOT ENTER).

- Centrifuging of samples must be done in a centrifuge with sealed buckets which are only opened in BSC and after 15-30 minutes.

- Laboratory staff must wear protective personnel equipment PPE. (see Appendix II)

- Staff must discard all PPEs (gloves, mask, gown…etc) in double biohazard bag upon leaving the DRA or completing their work with the samples. The bag should be sealed off for disposal

- Staff should perform hand hygiene after discarding the PPEs and after leaving the DRA. (see Appendix IX)

- All waste should be double bagged and the outer bag should be wiped with hypochlorite and should be sent for autoclaving and sterilization. (see Appendix V)
Other Laboratory areas – Precautions for handling non-inactivated samples:

**Hematology:**
Coagulations studies, DIC screening and CBC can be performed in closed sampling system analyzers. If de-capping of sample’s tube is done outside the analytical platform, then de-capping must be done inside the BSL-2 cabinet wearing the appropriate PPEs described above.

If closed system for coagulation is not available, then an experienced technologist in the DRA must perform coagulation studies using manual methods done inside the BSL-2 cabinet wearing the appropriate PPEs described above.

Decontamination cycles are performed immediately after analysis.

For Malaria screening, films made from the CBC sample can be fixed in methanol for 5 minutes and then in 10% buffered formalin for 15 minutes before stained & examined.

**Blood Bank:**
The current blood bank analyzer’s use an OPEN system and are therefore not suitable for grouping, antibody screening and cross-matching. Check previous transfusion history if possible.

If the patient requires blood, then Group O Rh negative packed cells (female patients of child bearing age) can be given. Male patients and female patients (not of child bearing age) may receive O Rh positive packed cells if Rh negative is not available or in short supply.

Single donor platelets, of any group, may be given. Group AB, FFP can be used. Cryoprecipitate of any group may be used when required.

The decision for compatibility testing should be discussed with consultant hematologist.

**Biochemistry:**
Samples for routine biochemical parameters can only be run directly if a closed sampling system is available as at Royal hospital otherwise the sample should be inactivated first. If de-capping of sample’s tube is done outside the analytical platform, then de-capping must be done inside the BSL-2 cabinet wearing the appropriate PPEs described above.

Please refer to the Inactivation procedure required for samples prior to analysis (Appendix I)

Adequate decontamination immediately post run may be performed.

Testing should be minimized to the following tests: U&E, LFTs, CRP, and glucose. For other parameters, please discuss with biochemistry laboratory prior to sending samples.
**Blood gases**

Wherever possible, these tests must be done on inactivated sera. If the method is not validated for this, then the test should be performed with manufacturer’s controls and the laboratory controls. Provided the control results are satisfactory, then the results may be utilized as provisional results. If VHF is subsequently excluded, then tests should be repeated on non-inactivated serum.

Non-inactivated specimens can be processed in automated analyzers that do not require removal of the top of the blood collection tube, provided there is proper disposal of waste fluids (see Appendix V) and the machine can be decontaminated after use.

**Microbiology:**
Testing **should not** be performed on any sample until it has been discussed with a Medical Microbiologist.

Blood cultures can be sent. Samples must be plated using disposable instruments in a BSL – 2 cabinet in the DRA wearing the appropriate PPEs. All primary cultures should be sealed and incubated in a CO2 incubator in the DRA. Secondary cultures may be handled in the routine laboratory.

Routine culture of non-sterile sites should be avoided. If needed, can be discussed with the responsible microbiologist.

**Virology:**
All samples for virology testing should be submitted to Central Public Health Laboratory (CPHL). All samples should be transported using triple packing system (see Appendix VI).

For samples required for virological diagnosis of suspected VHF, please see Appendix VII.

**Serology/ Immunology**
Testing should not be performed on any sample until it has discussed with a medical microbiologist. Provided the controls are satisfactory, then the results may be utilized as provisional results. If VHF is subsequently excluded, then repeat serology testing should be done on non-inactivated sera.

Immunofluorescence may be performed after fixation in 85%-100% acetone.

**Histopathology:**

**TESTS CANNOT BE PERFORMED ON UNFIXED TISSUE**
Testing **SHOULD NOT** be performed on any sample until it has been discussed with a Histopathologist. Specimens can only be processed after adequate fixation (volume of fixative is 10X the volume of the specimen) in 10% buffered Formalin or 2.5% Glutaraldehyde in the DRA. The specimen should be fixed for 48 hours and the tissue can be sectioned in the DRA by a suitably experienced scientist.

Frozen sections **MUST NOT** be performed.

Autopsies **MUST NOT** be performed

**Procedure for inactivation of samples:**

Heating at 60°C for 60 minutes for serum samples and other body fluids has been recommended by the CDC. This treatment does not significantly affect estimations of Na’ K⁺, Mg²⁺, urea, urate, creatinine, glucose, bilirubin and C reactive protein. Other parameters showed some variation whilst enzymes were inactivated.

This temperature is liable to coagulate IgG and invalidate serological tests. Based on experience with other viruses heating to 57°C for 60 minutes will provide sufficient viral inactivation and allow serological investigations.

For Malaria, air dried thick films should be fixed in 10% buffered formalin for 15 minutes then washed three times in distilled water pH 7.0 before staining. Thin films are fixed in absolute Methanol for 5 minutes then placed in 10% buffered formalin for 15 minutes then wash three times in distilled water pH 7.0 prior to standing.

Tissue samples may be fixed in 10% buffered formalin for sufficient time to fully penetrate the sample.

Inactivation of specimens can also be achieved by lysis procedures during nucleic acid extraction.

Swabs will be sufficiently inactivated after treatment with the lysis agent. Tissues may be fixed in 10% buffered formalin.

Specimens for immunofluorescent antigen detection are inactivated following fixation. Acetone (85-100%), 10% buffered formalin or 2.5% Glutaraldehyde for 15 minutes are satisfactory for inactivating the virus.
References:


3. CDC. Interim Guidance for Managing Patients with suspected Viral Hemorrhagic Fever in US Hospitals. 2005


APPENDIX I: Guidance for handling & inactivation of blood or fluids specimens for Biochemistry tests

The following guidance for handling blood or tissue fluids specimens for biochemistry tests from patients with suspected Viral Hemorrhagic Fever (VHF).

1- Blood specimens must be taken and transported to a designated receiving area in the laboratory with appropriate precautions.

2- The specimens have to be centrifuged in a biological safety cabinet (CLASS II).

3- The separated plasma or serum has to be heated at 60°C for 60min

4- The specimen will be subjected to the necessary biochemical analysis that has not been affected by the heating process.

5- Centrifuged tubes SHOULD NOT be opened for 15-30 minutes and should be opened in Class II biosafety cabinet. This to avoid exposure to aerosols.

This will reduce the risk for the laboratory staff and biochemistry analyzer will not require disinfection. The heating process will not affect certain tests and their results will be valid for interpretations that include: sodium, potassium, urea, creatinine, uric acid, total calcium, total bilirubin, glucose, magnesium and C-reactive protein. On the other hand, heating may partly affect other parameters hence interpretation has to be done with caution. Only elevated values of cardiac troponin and amylase are of diagnostic value, while levels within normal ranges for troponin and amylase do not exclude myocardial damage or acute pancreatitis respectively. Also, bicarbonate level within normal range usually excludes metabolic acidosis. The liver enzymes being protein in nature are markedly affected by heating and activities will decrease, however acute hepatocellular damage will be easily detected if the level enzyme results are high.
APPENDIX II: Infection control and Personal Protective Equipments (PPE)

Remember:
Specimens taken for laboratory analysis should be kept to the minimum necessary for patient management and diagnostic evaluation.

1. Specimen collection and transport:
The main risk of infection when collecting and handling specimens is direct contact with blood or body fluids from the patient, for example by accidental inoculation (needle stick) or contact with broken skin or mucous membranes.
Hence the Health Care Worker (HCW) should wear the appropriate PPE before entering the patient room and keep the Sharp bin ready in the trolley with other equipments he/she needs for collecting blood. In addition, universal infection control principles and practices should always be adopted.
After specimen collection, the entire outside surface of each specimen container should be wiped with disinfectant, and a label should be attached bearing the patient's name, hospital identification code, source of the specimen and date of collection. Laboratory specimens should be placed in plastic bags that are sealed, and then transported in durable, leak proof containers directly to the specimen handling area of the laboratory. Specimens should be transported by hand by a responsible person and submitted to laboratory personnel at the DRA (microbiology lab). Laboratory staff should be notified prior to receipt of all specimens from patients with suspected VHF.

2. Handling specimen in the laboratory:
For laboratory staff the potential exposure routes to be considered are direct contact (through broken skin or mucous membrane) with blood or body fluids, and indirect contact with environments contaminated with splashes or droplets of blood or body fluids.

PPE should be put on before starting procedures likely to cause exposure and only removed after moving away from a source of exposure.
The following PPE must be worn before handling specimen in DRA; the sequence of donning as follow:

1. Howie lab coat (see attached picture).
2. fluid resistant or impermeable gown
3. Fluid repellent surgical facemask (or N95 mask when performing aerosol generating procedures)
4. Eye protection (goggles or face shield)
5. Double Gloves
6. Overshoe boots must be worn in the event of decontaminating large spills on the floor.

After use, the work surfaces should be treated with 1,000ppm available chlorine (this should be left for at least two minutes before drying off).
Sequence of removing PPE:

1. Remove first the gloves
2. Wash your hand with soap and water
3. Then remove the gown.
4. Goggles or face shield
5. The last PPE to be removed is the surgical mask

Dispose the PPE in the yellow bag.

Hand washing should not be performed while wearing gloves or products such as alcohol based hand rub used to clean gloves as it may increase glove permeability.

PPE should not be a source of further contamination e.g., by being removed and left on environmental surfaces, or by being removed inappropriately thus contaminating the wearers hands.

- Offset front stud fastening
- Knitted cuffs
APPENDIX III: Accidental spills of potentially VHF contaminated material

1- If inactivated materials are spilled outside the BSC, evacuate the laboratory immediately for 15-60 minutes (depends on the amount of material spilled). This is to allow any potential aerosols to settle out

2- Close the door and put a no entry sign

3- Wear proper PPE before you re-enter the laboratory according to protocol, If required this should also include wearing disposable plastic overshoes (e.g. for large amount spilled in the floor)

4- Cover the area with disposable paper towels saturated with 1% sodium hypochlorite (bleach) {10,000ppm sodium hypochlorite = 1 part bleach to 4 parts water}.

5- Leave to soak 30 minutes then mop

6- Disinfect with 1% hypochlorite solution, leave for 2 minutes then wash with warm water and detergent. sodium dichloroisocyanurate (NaDCC) granules may be used - refer to manufactures’ instructions

7- Place the waste including gloves and paper towels in a double biohazard bags to be autoclaved or incinerated.
APPENDIX IV: Management of staff accidentally exposed to potentially infectious material

Accidental exposures that need to be dealt with promptly are:

**Percutaneous injury e.g. needlestick:**
Immediately wash the affected part with soap and water.

**Contact with broken skin:**
Immediately wash the affected part with soap and water.

**Contact with mucous membranes (eyes, nose, or mouth):**
Immediately irrigate the area with emergency wash bottles, which should be accessible in case of such an emergency.

In case of heavy contamination of clothing, the contaminated clothing must be discarded in the laboratory and the person should shower immediately.

In all cases, the incident will need to be reported (see Appendix VIII) and the individual referred urgently to the local Clinical Microbiologist or Infectious Disease Physician, and their occupational health provider.
APPENDIX V: Laboratory Waste Management

- All samples should be inactivated and autoclaved prior to disposal.

- Before transporting waste to a remote autoclave, arrangements to coordinate transport should be put in place.

- Waste should be contained within two layers of containment with the secondary containment being robust, leak-proof containers with a secure lid, transported on a trolley where appropriate. Autoclavable bags should be used as the primary containment.

- Waste should be transported direct to the autoclave for immediate treatment, thus avoiding storage in the autoclave room or in communal areas.

- Autoclave cycles must be appropriately validated to ensure that the required temperature and pressure conditions are reached for the appropriate length of time.

- After autoclaving, waste is no longer considered to be infectious, and can be disposed of via landfill or municipal incineration.
APPENDIX VI: Transport of samples

-Samples should be packed and labeled according to current regulations for Category A.

-The CPHL Laboratory should be notified when samples are dispatched.

-Packaging must meet with UN performance requirements i.e. UN-type approved packaging for Division 6.2 substances. The packaging should consist of an inner package (watertight receptacle, watertight secondary packaging, an absorbent material in sufficient quantity to absorb the entire contents placed between the receptacle and the secondary packaging) and a rigid outer package of adequate strength for capacity, mass and intended use.

- Packages should be marked with the proper shipping name (i.e. “Infectious substance affecting humans” UN 2814), and the appropriate warning label (i.e. the danger sign for infectious substances).

-The following procedures should be adopted for the transport of all specimens. These apply within hospitals and laboratories as well as for specimens sent to the reference laboratory:

-The primary container should be screwed tight, labeled and placed in an intact plastic bag.

- A ‘High Risk’ label should be affixed to both specimen and request form. The latter should include any other relevant information and include adequate clinical details to indicate level of suspicion.

- Under no circumstances should the request form be placed in the same bag as the specimen.

- The bag should be sealed, using tape or heat sealer. Pins, staples and metal clips should not be used. A separate bag should be used for each specimen.

-Each specimen must then be placed in a leak-proof secondary container with sufficient absorbent material to absorb all the contents should leakage occur.

Each specimen must be packaged individually - i.e. three specimens, three separate packages.

-The secondary container should be externally disinfected by wiping with hypochlorite (1,000ppm available chlorine).

Samples sent within hospitals and laboratories

-Secondary containers should be placed in a good quality box, which is well taped up and clearly labeled “Pathological Specimen – Open only in Laboratory”.

-Specimens should be transported by hand by a responsible person using the above packaging.

-Extra care should be taken to ensure that laboratory records are kept to a high Standard.
**Figure**: Triple Package for infectious substances

1. Primary receptacle (leakproof, 95kPa)
2. Secondary receptacle (leakproof)
3. Outer container (w/list of itemized contents)
APPENDIX VII: Sample required for virological diagnosis of suspected VHF

<table>
<thead>
<tr>
<th>Test name</th>
<th>Sample type</th>
<th>Sample volume</th>
<th>Collection times</th>
<th>Remarks</th>
</tr>
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</table>
| Molecular tests| EDTA blood                   | 5 ml blood (Two tubes) | Collect during acute phase (within 5-7 days of onset) | • Allow to settle for half an hour after collection in an erect position to avoid hemolysis  
  • Transport within 6 hours after collection  
  • Specimens anti-coagulated with heparin are not suitable for PCR |
| Serology tests | Serum (Clot Activator Tube)  | 5 ml blood (Two tubes) | Collect acute and convalescent (4 weeks after onset) sera |                                                                         |
APPENDIX VIII: Staff Record-Exposure to Suspected VHF Samples

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<th>Sultanate of Oman</th>
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<td>(Staff Record-Exposure</td>
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<td>Hospital ..........................</td>
<td>to suspected VHF Samples)</td>
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<tr>
<td>Department of Laboratory</td>
<td>Issue date:</td>
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<td>Consultant</td>
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<th>Date</th>
<th>Time:</th>
<th>*Shift (M, A, N):</th>
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Type of Samples

Tests Performed

Closed Sampling Systems used? Y/N

Inactivated sample used? Y/N

Problems encountered in performing the tests?

Samples returned to DRA for storage/disposal?

Decontamination procedure completed Y/N

State types of disinfectants used -

Probability of exposure after following standard precautions - Remote, low, Medium, High

Surveillance in ID indicated? Y/N

Ribavirin TM Prophylaxis indicated? Y/N

* Shift (M, A, N): Morning, Afternoon & night
**APPENDIX IX:** How to safely collect blood samples from persons suspected to be infected with highly infectious blood-borne pathogens e.g. VHF.

### Step 1: Before entering patient room, assemble all equipment

#### Step 1a: Assemble equipment for collecting blood:
- **Lab equipment**:
  - Blood sampling systems (Needle and syringe system, vacuum extraction system with holder, winged butterfly system (vacuum extraction) or winged butterfly system (syringe))
  - 2 X EDTA tubes (Purple top) 5ml/each for PCR.
  - 2 X serum tubes (Gel Separation tubes with clot activator), 5ml/each for serology
  - Blood tubes required for any other relevant investigations as indicated.

- **Disposable**:
  - Tourniquet (single-use)
  - Skin antiseptic wipes
  - Gauze pads
  - Disposable tray/dish for assembling blood collection tools
  - Adhesive bandage
  - Durable marker for writing on laboratory sample & Patient ID labels

#### Step 1b: Assemble equipment for preventing infection

**For Hand Hygiene**
- Clean, running water
- Soap
- Disposable (paper) towel
- Alcohol Hand rub

**Personal Protective Equipment (PPE):**
- Several pairs of disposable gloves
  - Two pair of gloves for blood collection
  - Two additional pairs as a replacement if they become damaged or contaminated
  - Footwear
    - Use disposable shoe covers secured around the shoes to prevent direct contact with ground and infected bodily fluid spills
  - Disinfectant wipes or spray (hypochlorite 1:100) to disinfect tubes and containers
  - Long-sleeved, cuffed water resistant / impermeable gowns
  - Face protection: Face shield or "goggles and mask"
**Step 1: Before entering patient room, assemble all equipment (continued...)**

**For waste management materials**
- Leak-proof and puncture resistant sharps container
- Two leak-proof yellow infectious waste bags

**Step 1c: Fill out patient documentation:**

- Label blood collection tubes with date of collection, patient name, and his/her identifier number.

- Do NOT forget to fill out necessary laboratory form and notification form.

**Step 1d: Assemble materials for packaging of samples:**

- Two plastic leak-proof packaging container or bags
- Disposable (paper) towels
- Cooler or cold box, if sample requires refrigeration

**Important:** A designated Assistant wearing gloves should be available for help. This person should stand outside the patient’s room. He/She will help you prepare the sample for transport, assist you with putting on the personal protective equipment, or provide any additional equipment you may need.
### Step 2: Put on all personal protective equipment (PPEs)

**DO NOT ENTER THE PATIENT AREA IF YOU DO NOT HAVE ALL PROTECTIVE GEAR ON**

**Step 2a:** Perform hand hygiene using alcohol hand rub or soap and water.

**Step 2b:** Put on a gown.

**Step 2c:** Put on face protection:

- **Put on a face shield**
- **Put on a medical mask and eye protection (e.g. eye visor/goggles)**

**Quick tips:** If the patient has respiratory symptoms, wear a medical mask underneath the face shield.

**Step 2d:** Put on double gloves (over gown cuffs).
### Step 3: Collect blood sample from patient

#### Step 3a: Prepare room.
- Put infectious waste bags and leak-proof and puncture resistant sharps container into patient room and make sure they are ready for use.
- Place all blood collection equipment in a place that is easy to access.

#### Step 3b: Identify and prepare the patient.
- Introduce yourself to the patient and explain what you will do with the blood sample and why.
- Make sure that this is the correct patient from whom you wish to take the blood sample.

#### Step 3c: Select the site, preferably at the bend of the elbow.
- Palpate the area; locate a vein of good size that is visible, straight and clear.
- The vein should be visible without applying a tourniquet.

#### Step 3d. Apply a tourniquet around the arm.
- Tie approximately 4–5 finger widths above the selected site.

#### Step 3e: Ask the patient to form a fist so that the veins are more prominent.

#### Step 3f: Disinfect the area where you will put the needle.
- Use 70% isopropyl alcohol wipes.
- Wait 30 seconds for the alcohol to dry.
- DO NOT touch the site once disinfected.

#### Step 3g: When using vacuum extraction system with holder, insert the blood collector tube into the holder.
- Avoid pushing the collector tube past the recessed line on the needle holder or you may release the vacuum.

#### Step 3h: Anchor the vein by holding the patient’s arm and placing a thumb BELOW the place where you want to place the needle.
- DO NOT touch the disinfected site.
- DO NOT place a finger over the vein to guide the needle

#### Step 3i: Perform the blood draw.
- Enter the vein swiftly at a 30° angle.
Step 3: Collect blood sample from patient (continued...)

**Step 3j:** When blood starts to flow, ask patient to open his/her hand.

**Step 3k:** Once sufficient blood has been collected (minimum 5ml), release the tourniquet **BEFORE** withdrawing the needle.

**Step 3l:** Withdraw the needle gently.
- Give the patient a clean gauze or dry cotton wool ball to press gently on the site.
- Ask the patient **NOT** to bend the arm.

**Step 3m:** Remove blood collector tube from holder and put into tray. **Do Not recap the needle.**

**Step 3n:** Put needle into leak-proof and puncture resistant sharps container.

**If the sharps container DOES NOT HAVE a needle remover:**
- Put the needle and holder into a sharps container.
- Do **not** remove the needle from the holder.
- Do **not** reuse the needle.

**If the sharps container HAS a needle remover:**
- Remove the needle following instructions on the sharps container.
- Put the holder into the yellow infectious waste bag for disinfection.

**Step 3o:** Stop the bleeding and clean the skin.
- Do **not** leave patient until bleeding has stopped.
- Put an adhesive bandage on the site, if necessary.

**Step 3p:** Put items that drip blood or have body fluids on them into double yellow waste bag for destruction.
### Step 4: Prepare blood sample for transport

#### Step 4a: Take the blood tube from the disposable tray & wipe blood tube with a disposable paper towel and disinfectant

![Image](image1.png)

#### Step 4b: Place all items that came into contact with blood into the infectious yellow waste bag for destruction.

![Image](image2.png)

#### Step 4d: Protect the sample from breaking during transport by wrapping the tube of blood in a paper towel and put it into the first plastic bag/leakproof container.

Disinfect the outer surface.

![Image](image3.png)

#### Step 4e: Ask the designated assistant to approach the patient room, without entering.

- This person should have double gloves on.
- This person should come close to you holding the open plastic leak-proof packaging container or open second plastic bag.
- This person should not enter the patient room.

![Image](image4.png)

#### Step 4f: The person who has collected the blood sample should put the wrapped tube of blood into the plastic leak-proof packaging container/bag.

- Be careful not to touch outside of leak-proof plastic tube with gloves.

![Image](image5.png)

#### Step 4g: Have the designated, gloved assistant tightly close the top of the plastic leak-proof packaging container/bag.

Disinfect the outer surface of the container/bag.

![Image](image6.png)
### Step 5: Remove Personal Protective Equipment (PPEs) inside the patient room

#### Step 5a: Remove the gloves.
1. Grasp the outer edge of the 1st glove and peel it off.
2. Hold the 1st glove in the gloved hand and drag a bare finger under the 2nd glove.
3. Remove 2nd glove from the inside, creating a “bag” for both gloves and throw it in waste bag for disposal.

#### Step 5b: Remove the gown
1. Untie the gown
2. Remove the gown from behind starting at the neck and shoulders.
3. Discard PPE into yellow bag.

#### Step 5c: Perform Hand hygiene with alcohol hand rub or soap & water if visibly soiled.

#### Step 5d: Take off face protection

**When wearing a face shield:**
- Remove face shield from behind & place it in the double yellow infectious waste bags.
- Optional: If wearing a medical mask, remove the medical mask from behind, starting with the bottom strap, and place it in a infectious waste bag for destruction.

**When wearing goggles and a mask:**
- Remove disposable goggles from behind and place in the double yellow infectious waste bag.
- Remove the medical mask from behind, starting with the bottom strap, and place it in a infectious waste bag for destruction.

#### Step 5e: Perform Hand hygiene with alcohol hand rub or soap and water if visibly soiled.
For laboratory personnel:

1. The laboratory should be notified in advance that samples from a case of SUSPECTED VHF are being sent.
2. A senior lab staff/consultant microbiologist should be informed and should liaise with attending clinicians on an agreed set of investigations.
3. ONLY ESSENTIAL INVESTIGATIONS SHOULD BE SENT TO THE LABORATORY UNTIL THE DIAGNOSIS IS VERIFIED AND VHF IS EXCLUDED.
4. The entire outside surface of each specimen container should be wiped with disinfectant, and a label should be attached bearing the patient’s name, hospital number source of the specimen, date of collection, and the nature of the suspected infection. Clinical laboratory specimens should be placed in plastic bags that are sealed, and then transported in durable, leak-proof containers directly to the specimen handling area of the laboratory.

For the shipment of samples to the Central Public Health Laboratory follow Sample Shipment packaging requirements (see the SOP for handling and management of VHF samples)

* Refer to MH/VHF/V1/Aug 2014

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Note: The sample is now ready to be shipped IMMEDIATELY to the Central Public Health Laboratory. Follow Sample Shipment packaging requirements for infectious substances.