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PARTNERS FOR HEALTH | PARTENAIRES POUR LA SANTÉ

Viral Haemorrhagic Fevers (VHFs) – Sample Collection and Submission Guide August 22, 2014

For information related to Ebola virus disease (EVD), please refer the PHO website at http://www.publichealthontario.ca/ebola

This document provides:

1. Background information on VHFs
2. Sample collection guidelines
3. Shipping instructions
4. Links for further information

1. Background

Viral haemorrhagic fevers (VHFs) are characterized by initial non-specific symptoms, including acute onset of fever, myalgia, headache, pharyngitis, diarrhoea, and chest pain. Later signs are more specific to VHF including conjunctivitis, petechiae and morbilliform rash with possible progression to haemorrhagic shock. In severe and fatal forms, the haemorrhagic diathesis may be accompanied by hepatic damage, renal failure, CNS involvement and terminal shock with multi-organ dysfunction.

VHFs are associated with a number of geographically restricted viruses including **Lassa fever, Marburg virus haemorrhagic fever, Ebola Virus Disease (formerly Ebola haemorrhagic fever), Crimean-Congo haemorrhagic fever, Bolivian haemorrhagic fever (Machupo) and Venezuelan haemorrhagic fever (Guanarito).**

VHFs are not indigenous to Canada, but there have been both suspected and confirmed convalescent cases, and the potential for importation of an acute case is of concern. Circumstances under which the diagnosis of acute VHF should be considered are individuals who, within 3 weeks before onset of fever, have either:

- travelled in the specific local area of a country where VHF has recently occurred (if exact travel history is unknown, risk assessment should be done through consultation with an Infectious Disease Specialist).
- had direct contact with blood, other body fluids, secretions, or excretions of a person or animal with VHF, or
- worked in a laboratory or animal facility that handles haemorrhagic fever viruses.

VHFs are known to have caused outbreaks of disease with person-to-person spread . Transmission of VHF from person to person is primarily due to contact with virus-infected blood and body fluids, such as urine, vomitus or feces, or by the use of contaminated needles or syringes in the health care setting. Ebola, Marburg and Lassa viruses can be transmitted by semen for up to 3 months after clinical recovery. Epidemiological studies of VHF in humans indicate that infection is not transmitted readily from person to person by the airborne route. Airborne transmission involving humans is considered a possibility only in rare instances from persons with advanced stages of disease and pulmonary involvement. The risk for person-to-person transmission of a haemorrhagic fever virus is highest during the later stages of illness, which are characterized by vomiting, diarrhoea, shock and often haemorrhage.

It is important that in all suspected cases, other more common and potentially treatable diseases such as malaria are eliminated from the differential diagnosis.

When a possible case of VHF is suspected, the following tests must be done immediately:

- Blood film examination for **malaria (thick and thin blood films)**; a smear from a second specimen must be examined 12 to 24 hours later if the first does not reveal parasites.
- Two sets of **blood cultures** with a total volume per set (two bottles) of 16 to 20 ml in adults. Recommended volume collection for blood cultures in children is based on body weight.
- White blood cell and differential counts, and either haemoglobin or haematocrit.urine culture, if symptoms or manual dipstick suggests infection. Other critical tests can be considered if they can be performed on closed systems, i.e. arterial blood gases, electrolytes, liver function tests, creatinine, clotting function.

2. Specimen Collection Guidelines

Before the collection of specimens to be submitted to PHO Laboratory (PHOL), contact PHOL Customer Service Centre at 416-235-6556 or 1-877-604-4567. The following five principles should be observed in the collection of all patient specimens:

- Only specimens essential for diagnosis or monitoring should be obtained.

- Specimens should be obtained by staff experienced in the required techniques. The same protective clothing as described for other hospital staff should be worn by those obtaining and testing laboratory specimens.

- Glass containers should **not** be used. Disposable sharp objects, such as scalpel blades, should be placed in approved sharps containers immediately after use and later autoclaved or incinerated before disposal.

- Blood samples must be collected with extreme care to avoid self-inoculation. Needles should not be removed from disposable syringes, or otherwise handled. After use, blood-taking equipment should be immediately placed in approved sharps disposal containers and autoclaved or incinerated before disposal.

- 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, date of collection, and the nature of the suspected infection**. Clinical laboratory specimens should **each** be placed into a separate **sealable plastic biohazard bags** that are sealed, then transported in a **durable, leak-proof secondary container** directly to the specimen handling area of the laboratory. A fully completed laboratory requisition form for each sample should be placed in a separate pocket of the biohazard bag, **not** inside the sealed compartment with the sample. The outside of these biohazard bags should be wiped with a disinfectant solution such as a 1:10 dilution of household bleach (5,000 ppm available chlorine) before leaving the patient's room. **Automated delivery (pneumatic tube) systems should not be used** as they may disseminate aerosols in the event of a spill or breakage. **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.

Table 1: Recommended specimen collection guidelines for diagnosis/detection of specific viral aetiology of VHF.

Specimen	Test	How to submit
Blood	Serology	Two (2) serum separator tubes (SST) (Ideally 10ml each; minimum 2ml each)
Blood	Viral culture PCR	Two (2) tubes containing EDTA (Ideally 10ml each, minimum 2ml each)
Throat swab	Viral culture PCR	Place swab in plastic screw cap container with Viral Transport Media (VTM)
Tissue[µ]	Viral culture PCR	Place in sterile screw cap container.

µ Tissue should not be tested as a first line specimen due to biosafety concerns related to specimen collection.

- Each sample for VHF-specific testing should be submitted with its own separate requisition form requesting VHF testing only – specify which particular VHFs are suspected. Other tests requested on same requisition will be cancelled.
- If additional tests are requested submit separate samples, each with its own laboratory requisition, **clearly stating patient's suspected diagnosis and risk factors**. Other tests may be delayed pending VHF testing results.

3. Shipping Instructions

- Shipping of samples must be done in accordance with the Transportation of Dangerous Goods Regulations (TDGR) by a TDG certified individual. These regulations require handling and shipping patient's samples according to the international procedures for transport of category A infectious substances (UN2814). See link provided below for further information.
- Store samples in refrigerator or frozen until being shipped for testing.
- Frozen specimens should be shipped on dry ice and refrigerated samples with ice-packs. Blood and throat swabs should be shipped immediately with cool packs. If shipment is expected to take longer than 24 hours it should be shipped on dry ice. Tissue should be preferentially shipped on dry ice.
- **Ship specimens in separate sealable leak-proof biohazard bags** placed in an approved shipping container.

4. Specimen Handling/Processing in the Laboratory

- **Process clinical specimens for microbiology testing, including malaria smears, in a class II biological safety cabinet following biosafety level 3 practices.**

- Heating at 60°C for one hour renders specimens non-infectious and enables measurement of heat-stable substances such as electrolytes, blood urea nitrogen, and creatinine.
- Non-inactivated specimens can be processed in automated analysers that do not require removal of the top of the blood collection tube, provided there is proper disposal of waste fluids and the machine can be decontaminated after use.
- Haematologic specimens can be processed in automated analyzers provided it does not require manual removal of the top of the blood collection tube and there is proper disposal of waste fluids.
- Blood smears (for malaria, blood films) are not infectious for VHF viruses after fixation in solvents.
- Specimens for nucleic acid amplification can be inactivated by heat treatment at 60°C for one hour. Inactivation also occurs once material is exposed to the lysis reagent used for nucleic acid extraction e.g. guanidinium thiocyanate.
- **Note: Cross-matching of blood cannot be performed safely.** If transfusion is required, O Rh negative blood (universal donor) should be used.

See section 5 (second bullet) for links to relevant documents with further information on methods for viral inactivation and how to conduct non-microbiological essential laboratory testing.

5. Further Information

- Contact the PHOL Customer Service Centre at 416-235-6556 or 1-877-604-4567 (toll-free).
- For further information about the processing of specimens of suspect or confirmed case with Viral Haemorrhagic Fever (VHF) in hospital laboratories:
    - CDC: Interim Guidance for Managing Patients with Suspected Viral Haemorrhagic Fever in U.S. Hospitals
    - Australian Public Health Laboratory Network: Laboratory Precautions for Samples Collected from Patients with Suspected Viral Haemorrhagic Fevers
- PHOL: Laboratory services and testing information
- PHOL: Viral Haemorrhagic Fever Testing Information Sheet
- The current version of the PHOL laboratory requisition form is available here.
- PHAC: Viral Haemorrhagic Fever homepage
- Biosafety information relevant to viral haemorrhagic fevers (PHAC)
    - PHAC: Pathogen Safety Data Sheets and Risk Assessment (index)
    - PHAC: Canadian Biosafety Standards and Guidelines –First Edition
- Transport Canada : Transportation of Dangerous Goods Regulations