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LABORATORY GUIDANCE

Viral Haemorrhagic Fevers including Ebola Virus Disease

About this Document

This document provides:

- Testing recommendations for cases of suspected viral haemorrhagic fevers (VHFs) including Ebola virus disease (EVD)
- Specimen collection guidelines
- Specimen handling and processing guidance for the laboratory
- Further information

For additional information on VHFs and EVD, please visit Public Health Ontario's (PHO) Viral Hemorrhagic Fevers page.

This document does not provide information about dengue virus testing or yellow fever virus testing. For information on those viruses see the Dengue Virus Test Information Sheet and Yellow Fever Virus Test Information Sheet.

1. Testing Recommendations for Cases of Suspected Viral Haemorrhagic Fever Including Ebola Virus Disease

VHFs Including EVD

VHFs, including EVD, should be initially suspected in all patients with fever and a relevant travel history or epidemiological exposure within 21 days prior to illness onset. A relevant travel history includes travel to any geographic area where VHF outbreaks are occurring, including EVD outbreaks.

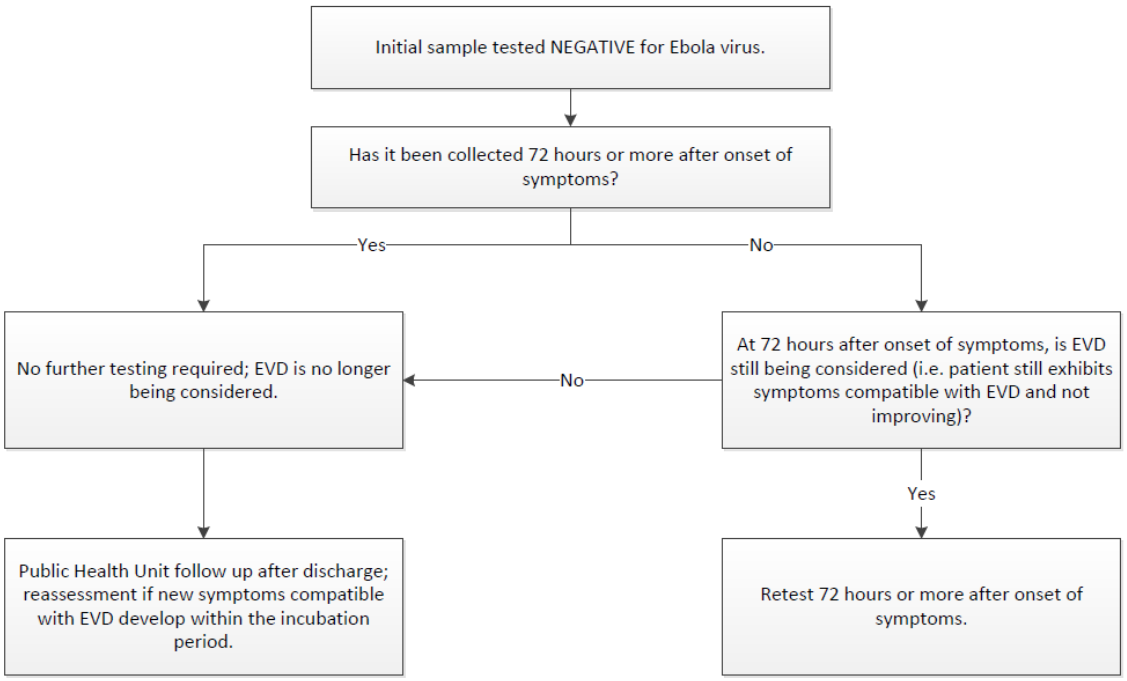
Additionally, VHFs should be suspected in patients with a compatible clinical illness who have travelled within 21 days to any country where sporadic cases of VHF occur.

- Please note: Ebola virus RNA is detected in blood only after onset of symptoms and it may take up to 72 hours after symptom onset for the virus to reach detectable levels. If a negative result is received on a specimen taken at least 72 hours after symptom onset, no further Ebola testing is needed.
- If a negative test result is received from a specimen that was taken less than 72 hours after symptom onset, a second specimen collected 72 hours or more after symptom onset should be tested, ***but only if Ebola is still suspected on reassessment of the patient (e.g. in patients who***

are not improving clinically at 72 hours after symptom onset). If a second test is ordered, the patient should remain in isolation in hospital with the staff using appropriate Ebola precautions until the second negative test result is received. See Figure 1.

- Patients at risk for Ebola often develop fevers from other causes (e.g. malaria, typhoid, and viral illnesses). If a person under investigation recovers after testing negative for Ebola, then subsequently develops a new illness compatible with Ebola within 21 days since the last date of potential exposure, they should be re-evaluated for possible EVD, as the initial febrile illness may have been due to another cause.

Figure 1: Decision tree for repeat testing for Ebola virus 72 hours after symptom onset



A clinical assessment of risk of VHF, including risk factors of exposure, clinical status and consideration of differential diagnoses is required prior to requesting VHF testing.

The decision to proceed with VHF testing requires the concurrence of a PHO Microbiologist.

For detailed instructions about how to request VHF testing and submission overview, see Laboratory Guidance – Specimens requiring Emergency Response Assistance Plan (ERAP) for transport within Canada

As of April 29, 2019, *Zaire ebolavirus*, *Bundibugyo ebolavirus, Crimean-Congo hemorrhagic fever virus, Lassa fever virus, Marburg virus* and *Rift Valley fever virus* PCR testing is performed by PHO laboratory. Depending on the prevalence and of a particular virus at the time of testing, and the clinical features of the individual case, negative test results may be reported as final by PHO laboratory, or alternately be retested at NML for final reporting. The reporting plan will be communicated to stakeholders at the time of testing. In accordance with federal guidelines, positive or indeterminate results are provisional until confirmed by the National Microbiology Laboratory (NML).

VHF real-time PCR tests are performed using a protocol validated at NML, and verified for clinical testing at PHO laboratory. The protocol tests for two gene targets per viral agent: *Zaire ebolavirus*, *Bundibugyo ebolavirus, Crimean-Congo hemorrhagic fever virus, Lassa fever virus, Marburg virus* and *Rift Valley fever virus*. Laboratory confirmation requires detection of both targets, with confirmation by NML. Specimens with both targets detected are reported as e.g. *Zaire ebolavirus* RNA detected by PHO laboratory. If a single target is detected, or testing is indeterminate for either target, testing is also repeated at NML. All positive and indeterminate PHO laboratory results are provisional until confirmed by NML.

For information on laboratory confirmation of viral haemorrhagic fevers, see section 4.0 of Ontario Ministry of Health and Long-Term Care Infectious Disease Protocol Appendix 1: Hemorrhagic fevers.[1]

Further information about laboratory testing and clinical management can be found here:

- PHAC: National Case Definition: Ebola Virus Disease (EVD)[2]
- CDC: Considerations for Discharging People Under Investigation (PUIs) for Ebola Virus Disease (EVD)[3]
- WHO: Laboratory Diagnosis of Ebola Virus Disease[4]

Other Testing:

It is important that other more common and potentially fatal diseases including malaria, typhoid fever and bacteremia are considered in the differential diagnosis of patients presenting with suspected VHF.

Co-infection with Ebola virus and malaria, as well as other pathogens, has been described.

Once there is a consensus that the patient meets criteria as a suspect case of EVD/ VHF, the following testing should be performed urgently.

1. Examination for malaria

- Testing may include thin smears, immunochromatographic (ICT)/ rapid tests or PCR.
    - Testing for malaria is available at PHO laboratory*.
    - For malaria testing to be performed at PHO laboratory, collect a minimum of 2 ml of blood (1 ml for infants) in a lavender top (EDTA) tube.
    - Do not send pre-prepared malaria smears to PHO laboratory.

* Specimens for malaria testing collected from suspected or confirmed VHF cases that are subject to Part 7 of the Transport Canada Transportation of Dangerous Goods (TDG) regulation, *Emergency*

Response Assistance Plan (*ERAP*), require special shipping and handling. For more information about shipping, including a link to TDG regulations, see Laboratory Guidance – Specimens requiring Emergency Response Assistance Plan (ERAP) for transport within Canada [link when available]

2. Other essential testing not offered at PHO laboratory includes:

- Two sets of blood cultures.
- Complete blood count, INR, PTT, electrolytes, creatinine, transaminases, glucose.

Testing that should be avoided until viral haemorrhagic fevers have been excluded includes:

- Cross-matching of blood - cannot be performed safely. If transfusion is required, type O Rh negative blood (universal donor) should be used.
- Cultures of non-sterile sites, and testing for influenza and other respiratory viruses (as they are non-essential for acute management).

2. Specimen Collection Guidelines

This section applies to **all** specimens collected from a patient with suspected VHF.

Prior to any specimen collection, discuss with your local laboratory management to ensure that any specimens for testing are collected and transported appropriately and testing is performed safely.

Key Specimen Collection Guidance

The following should be observed in the collection of **all** specimens from patients suspected to have VHF:

• Only specimens essential for diagnosis or monitoring should be collected.

• Specimens should be obtained by staff experienced in the required techniques.

• Follow recommended safety procedures including proper use of personal protective equipment (PPE).

• Laboratory staff should be alerted to the nature of the specimens which, once received, should remain in the custody of designated persons from the time of specimen receipt until testing is complete and specimens are safely discarded.

Specimens to Collect for VHF (including EVD) and Malaria Testing

Table 1: Recommended Specimen Collection Guidelines for Diagnosis/Detection of Ebola Virus, other VHF Agents and Malaria.

Test	Specimen
Real-time PCR for *Zaire ebolavirus* and /or other viral agents causing viral haemorrhagic fevers[Δ]	**TWO TUBES ARE REQUIRED:** 2 to 4 ml whole blood in each of two EDTA containing tubes[∏*$]
Malaria rapid test, thin smear and real-time PCR	**ONE ADDITIONAL TUBE IS REQUIRED:** 2 to 4 ml whole blood in one EDTA containing tube[∏*$]

Δ As clinically and epidemiologically indicated.

∏ 1 ml in each tube is sufficient volume for an infant or if specimen collection is difficult.

* Tubes should not be opened or pretreated prior to transport.

$ Malaria testing requires a separate tube. DO NOT submit pre-made thick and thin smear slides on patients under investigation for VHF/EVD.

Additional Guidance

- Do not use glass specimen collection devices/containers, unless there is no other alternative.
- Automated delivery (pneumatic tube) systems should NOT be used as they may disseminate aerosols in the event of a spill or breakage.
- Collect the appropriate number of specimens for testing and label with a minimum of two patient identifiers. PHO laboratory will not aliquot samples under investigation for ERAP agents
- Each specimen for VHF/EVD testing submitted to PHO laboratory should be submitted with its own separate PHO laboratory General Test Requisition, requesting only EVD/VHF testing – specify which particular VHFs testing has been arranged for. Non-VHF/EVD tests requested on the same requisition will be cancelled.
- If additional tests such as malaria are requested of PHO laboratory, separate specimens must be submitted, each with its own PHO laboratory General Test Requisition, **clearly stating patient's suspected diagnosis and risk factors**. Non-essential microbiology tests sent to PHO laboratory will be postponed pending VHF/EVD testing results.

3. Specimen Handling and Processing Guidance for the Laboratory

Enhanced Precautions

- Refer to the Public Health Agency of Canada's (PHAC) Biosafety Guidelines for Laboratories Handling Specimens from Patients under Investigation for EVD.[5]

Cross-Matching

- **Cross-matching of blood cannot be performed safely**. If transfusion is required, O Rh negative blood (universal donor) should be used.

Pre-treatment of Specimens

- **All pre-treatment and manipulation should occur within a certified class II BSC with enhanced precautions for laboratory testing described above.**

- Pre-treatment of specimens reduces the titre of Ebola virus and may facilitate the measurement of substances in non-closed systems. As recommended by the CDC, pre-treatment of serum can be achieved with the combination of "heat-inactivation at 56° C and polyethylene glycol p-tert-octylphenyl ether (Triton™ X-100)*; treatment with 10 uL of 10% Triton™ X-100 per 1 mL of serum for 1 hour reduces the titer of hemorrhagic fever viruses in serum, although 100% efficacy in inactivating these viruses should not be assumed." The CDC document also states: "Heat inactivation alone may be of some benefit in reducing infectivity." (Interim Guidance for Managing Patients with Suspected Viral Haemorrhagic Fever in U.S. Hospitals,[6] accessed January 22, 2022).

- If using heat pre-treatment alone, heating for one hour at 60◦C is recommended.[7] This renders specimens non-infectious and enables measurement of heat-stable substances such as electrolytes, blood urea nitrogen, and creatinine.

- Pre-treatment is also achieved by lysis procedures used for nucleic acid extraction; e.g., guanidinium thiocyanate.

- Thin blood smears (for malaria, blood films) are not infectious for VHF viruses after standard fixation in methanol.

Use of Analyzers for Testing

- All specimen handling, from the accessioning window through to analysis within an automated system, should be done wearing full PPE as described above, and any manipulation of the specimen, including the removal of the cap, should be done in a Class II BSC.

- Non-inactivated specimens can be processed for haematologic and biochemical testing in automated analyzers that are closed systems and 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.

- If closed systems for haematology and chemistry testing are not available, you should discuss testing with the core laboratory director.
- All waste including specimen tubes, cuvettes and other liquid or solid waste should always be disposed of safely as biohazardous waste.
- Routine cleaning and disinfecting procedures after use can be used for automated analyzers as recommended by the manufacturer.

For additional information about the processing of specimens from a suspect or confirmed case of Viral Haemorrhagic Fever (VHF) including Ebola (EVD) in hospital laboratories:

- CDC: Guidance for Collection, Transport and Submission of Specimens for Ebola Virus Testing[8]
- CDC: Interim Guidance for Managing Patients with Suspected Viral Haemorrhagic Fever in U.S. Hospitals[6]
- Australian Public Health Laboratory Network: PHLN laboratory procedures and precautions for samples collected from patients with viral haemorrhagic fevers[9]

4. Further Information

For further information about testing of specimens for VHFs including EVD and other VHF/EVD information:

Ministry of Health and Long-Term Care

- Emergency Management: Ebola Virus Disease

Public Health Ontario

- Ebola Virus Disease (EVD) web page
- PHO laboratory services and testing information
- Viral Haemorrhagic Fever including Ebola Virus Disease Testing Information Sheet
- PHO laboratory General Test Requisition

Public Health Agency of Canada: biosafety information relevant to viral haemorrhagic fevers

- Pathogen Safety Data Sheets and Risk Assessment (index)
- Canadian Biosafety Standards and Guidelines – Second Edition
- Biosafety Guidelines for Laboratories Handling Specimens from Patients Under Investigation for Ebola Virus Disease

Transport Canada

- Transportation of Dangerous Goods Regulations
- TDG Infectious Substance Bulletin

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