Public Health Ontario | Santé publique Ontario

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)
- Process for requesting testing for VHF including EVD
- Specimen collection guidelines
- Specimen submission overview
- Specimen handling and processing guidance for the laboratory
- Further information

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

This document does not provide information about dengue virus testing or yellow fever virus testing. See the Dengue Virus Test Information Sheet or 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.

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 Laboratory Microbiologist.

For detailed instructions about how to request VHF testing, including Ebola, see Section 2: Process for Requesting Testing for VHF Including EVD.

As of April 29, 2019, *Zaire ebolavirus* PCR testing is performed at PHOL Toronto. Negative test results are reported as final. In accordance with federal guidelines, positive or indeterminate results are provisional until confirmed by the National Microbiology Laboratory (NML).

All other VHF testing is performed at NML.

Zaire ebolavirus real-time PCR is performed using a protocol validated at NML, and verified for clinical testing at PHOL. The protocol tests for two gene targets, the polymerase (L) and nucleoprotein (NP). Laboratory confirmation requires detection of both targets, with confirmation by NML. Specimens with both targets detected are reported as *Zaire ebolavirus* RNA detected by PHOL. If a single target is detected, or testing is indeterminate for either target, testing is also repeated at NML. All positive and indeterminate PHOL 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 B: Hemorrhagic fevers.

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

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

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.

a) Examination for malaria

- Testing may include thin smears, immunochromatographic (ICT)/ rapid tests or PCR.
    - Testing for malaria is available at PHO's laboratory*.

- For malaria testing to be performed at PHO's 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's 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 Section 4: Specimen Submission Overview.

b) Other essential testing includes:

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

Testing that should be avoided until Ebola virus disease has 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. PROCESS FOR REQUESTING TESTING FOR VHF INCLUDING EVD

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

Before Collecting Specimens

When a viral haemorrhagic fever (VHF), including Ebola Virus Disease (EVD) is suspected, immediately

- **Inform:**
  - your local/hospital infection prevention and control team, occupational health and safety team and an infectious diseases specialist
  - your local/hospital laboratory management and microbiologist
  - your local public health unit
- **Obtain:** a clinical assessment of VHF risk, including risk factors of exposure, clinical status and consideration of differential diagnoses. **This is required prior to requesting VHF testing**.

- Refer to Section 3: Testing Recommendations for VHF, including EVD.
- If there is still a concern of VHF infection then:
    - Contact PHO's laboratory to request VHF/EVD testing.
    - Do not collect specimens for microbiology testing before consulting with the PHO Laboratory Microbiologist.

Process for Requesting VHF/EVD testing from PHO's Laboratory

To initiate VHF testing, the hospital contacts PHO's laboratory's Customer Service Centre at 416-235-6556 or 1-877-604-4567 (Monday to Friday 7:30 a.m. – 7 p.m. and Saturday 8 a.m. – 3:45 p.m.), or the PHO Laboratory Duty Officer after-hours at 416-605-3113. A PHO Laboratory Microbiologist and designated staff will follow up with the patient care team.

After the PHO Laboratory Microbiologist concurs that VHF testing is appropriate, PHO's laboratory will follow up with the submitter about next steps.

3. 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 obtained.

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

- Do not use glass specimen collection devices/containers, unless there is no other alternative.

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

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[∏*$]
Malaria rapid test, thin smear and real-time PCR	**ONE TUBE IS REQUIRED:** 2 to 4 ml whole blood in one EDTA containing tube[∏*$]

Δ As clinically and epidemiologically indicated.

∏ 1 ml 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 thin smear slides on patients under investigation for VHF/EVD.

Additional Guidance

- Follow institutional and provincial guidelines as well as Transport Canada's Transportation of Dangerous Goods Regulations (TDGR), including their Shipping Infectious Substance Bulletin for specimen collection, transportation and storage.
- **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, once received, should remain in the custody of designated persons from the time of specimen receipt until testing is complete.
- Aliquotting of specimens collected for VHF/EVD testing should be avoided if at all possible.
- Each specimen for VHF/EVD testing submitted to PHO Laboratory should be submitted with its own separate PHO's 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.

4. Specimen Submission Overview

Communications Plan

If VHF/EVD testing is agreed to, PHO's laboratory would like to have as contacts:

- A member of the patient's clinical management team or designate.
- The TDG-certified person responsible for shipping.

PHO's laboratory will assign designated staff who will function as key contacts and maintain contact with the consignor during the shipment process. Should an issue arise during transportation PHOL may be able to offer advice or otherwise provide assistance.

Shipping to an External Testing Laboratory

> This overview is not intended to replace or supersede Transport Canada's Transportation of Dangerous Goods Regulations (TDGR). Always follow the most up-to-date TDGR. Transportation of Dangerous Goods (TDG) consignors (formerly "shippers") must be certified.
>
> Note: this shipping process does not apply to specimens submitted for dengue virus testing or yellow fever virus testing when there is no concern of illness due to other VHFs. For more information about dengue virus testing and yellow fever virus testing see the Dengue Virus Test Information Sheet and Yellow Fever Virus Test Information Sheet.

1. **Activation of ERAP**

   a) After a PHO Laboratory Microbiologist has concurred to test the patient for VHF/EVD, the consignor contacts PHO's laboratory for facilitation of the ERAP process for VHF/EVD and malaria testing with the ERAP holder.

   b) The recipient laboratory(s) provides the consignor with their shipping address, the name of the consignee and a phone number that will be answered by a person with knowledge of the consignment if there is a problem, as required for an ERAP shipment.

   c) Only one ERAP activation is required at a time for one or a group of consignments, beginning when the first consignment is picked up and ending when the last consignment is delivered, providing that at least one of the consignments is always in transit.

2. **Preparation of consignment**

NOTE: The following is **only an overview**. It is the responsibility of the TDG certified consignor to ensure that TDGR regulations are met in the preparation of packages, shipping documents and other aspects of shipping regulated by TDGR.

   a) Make arrangements with a carrier who is TDG certified and able to transport an ERAP agent.

b) Prepare packages for shipment:

i) Specimens must be packaged in type P620 packaging (formerly known as a "type 1A packaging") as explained in the link.

ii) To facilitate purchase of type P620 packaging, a list of vendors can be accessed on the Government of Canada's website.

iii) Specimens must be packaged with cold packs which can be placed between the primary and secondary container. Do not ship whole blood on dry ice; NEVER place dry ice within sealed, pressure-resistant containers.

c) Prepare the shipping documents for the shipment:

i) For land transportation, use a **Shipping Document for Surface Transport**. Each shipping site develops their own document.

ii) If transporting by air, use a **Shipper's Declaration for Dangerous Goods**.

- This document is carrier specific and is available from each carrier on their website.
- The document must be printed in colour.

iii) Waybill (obtained from the carrier at time of shipment).

iv) The ERAP reference number must be included where indicated on the shipping documents.

d) Be prepared to provide four Class 6.2 vehicle placards (10.75in X 10.75in) per carrier vehicle in case the carrier does not have them available. These are displayed on 4 sides of the vehicle transporting the ERAP consignment. Placards should have UN2814 printed on the centre. Placards may be purchased from suppliers of dangerous good supplies in preparation for a potential suspect case.

e) Ship the packages:

i) Inform the ERAP holder (see 1a above) when the consignment(s) are being picked up and they will activate the ERAP.

ii) Each consignee (e.g. the receiving laboratory) informs the ERAP holder when their consignment is received.

iii) When the ERAP holder has been notified that the last consignment has been received, they notify the consignor and all consignees that the ERAP is deactivated.

3. Response to issues arising during transportation

a) Response to spills: in the event of a spill during transport, immediately call:

i) CANUTEC at 1-888-CAN-UTEC (226-8832), 613-996-6666 or *666 on a cellular phone.

The consignor must be registered with CANUTEC.

ii) NML OCD at 1-866-262-8433.

b) Response to other issues:

Should other issues arise during transportation, depending on the nature of the issue, PHO's laboratory may be able to offer advice or otherwise assist. Contact the PHO's laboratory designated staff assigned to this response (see Communications Plan above), or PHO's laboratory Customer Service Centre at 416-235-6556 or 1-877-604-4567 (Monday to Friday 7:30 a.m. – 7 p.m. and Saturday 8 a.m. – 3:45 p.m.), or the PHO Laboratory Duty Officer after-hours 416-605-3113.

5. 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.

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

- Pretreatment of specimens reduces the titer of Ebola virus and may facilitate the measurement of substances in non-closed systems. As recommended by the CDC, pretreatment of serum can be achieved with the combination of "heat-inactivation at 56° C and polyethylene glycol p-tert-octylphenyl ether (TritonTM X-100)*; treatment with 10 uL of 10% TritonTM 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, accessed April 30, 2019)

- If using heat pre-treatment alone, heating for one hour at 60oC is recommended (Mitchell SW and McCormick JB, .J Clin. Microbiol. 1984, 20(3):486.). 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 of 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
- CDC: Interim Guidance for Managing Patients with Suspected Viral Haemorrhagic Fever in U.S. Hospitals
- Australian Public Health Laboratory Network: Laboratory Precautions for Specimens Collected from Patients with Suspected Viral Haemorrhagic Fevers

6. Further Information

For further information about testing of specimens for Viral Haemorrhagic Fevers (VHF) including Ebola (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's Laboratory Services and testing information
- Viral Haemorrhagic Fever including Ebola Virus Disease Testing Information Sheet
- PHO's 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 – First 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

Disclaimer

This document was developed by Public Health Ontario (PHO). PHO provides scientific and technical advice to Ontario's government, public health organizations and health care providers. PHO's work is guided by the current best available evidence at the time of publication.

The application and use of this document is the responsibility of the user. PHO assumes no liability resulting from any such application or use.

Public Health Ontario

Public Health Ontario is a Crown corporation dedicated to protecting and promoting the health of all Ontarians and reducing inequities in health. Public Health Ontario links public health practitioners, frontline health workers and researchers to the best scientific intelligence and knowledge from around the world.

Public Health Ontario provides expert scientific and technical support to government, local public health units and health care providers relating to the following:

- communicable and infectious diseases
- infection prevention and control
- environmental and occupational health
- emergency preparedness
- health promotion, chronic disease and injury prevention
- public health laboratory services

Public Health Ontario's work also includes surveillance, epidemiology, research, professional development and knowledge services. For more information, visit publichealthontario.ca.

Public Health Ontario acknowledges the financial support of the Ontario Government.

Ontario
Agency for Health Protection and Promotion
Agence de protection et de promotion de la santé