Ebola Virus Disease (EVD) Interim Sample Collection and Submission Guide

July 29, 2015

This document has been updated as of July 29, 2015, based on the best available evidence at that time. Version changes are summarized at the end of this document. Please refer to the Public Health Ontario website, at www.publichealthontario.ca/ebola for the most recent version.

THIS DOCUMENT PROVIDES:

1. Clinical presentation of Ebola virus disease
2. Testing recommendations for cases of suspected Ebola virus disease
3. Sample collection guidelines
4. Shipping instructions
5. Specimen handling/processing in the laboratory
6. Links for further information

1. CLINICAL PRESENTATION OF EBOLA VIRUS DISEASE

Ebola virus disease (EVD) is a severe illness that starts with the abrupt onset of fever, usually with headache, malaise and myalgia. Gastrointestinal symptoms (i.e., diarrhea, abdominal pain, vomiting) are common. Additional symptoms and signs may occur (e.g., sore throat, chest pain, cough, rash, conjunctivitis). Hemorrhagic findings (e.g., petechiae, ecchymosis, and hemorrhage) occur in a minority of cases. Leukopenia, thrombocytopenia and transaminitis (elevated liver enzymes) are common laboratory findings. The case fatality rate ranges from 50 to 90 per cent. However, outbreaks have often occurred in areas where the capacity for supportive care is limited and therefore, case fatality rates in well-resourced healthcare systems remain undetermined.

The incubation period for EVD is 2 to 21 days. Patients are not infectious during the incubation period and prior to the onset of symptoms. Person-to-person transmission can occur, primarily through direct contact with blood, body fluids, secretions and excretions of someone who is sick, or through indirect contact with material contaminated with these substances. Ebola virus is not an airborne pathogen. Transmission of EVD during the incubation period while the person is still well has not been reported.

Outbreaks of EVD have been reported periodically in several central African countries. In March 2014 the first reports of an EVD outbreak in West Africa were received by the World Health Organization (WHO). See the WHO’s Global Alert and Response Website on EVD at http://www.who.int/csr/don/archive/disease/ebola/en/ for the latest details of the outbreak. This is the largest EVD outbreak ever identified. While sporadic cases of EVD and other viral hemorrhagic fevers (VHF) should always be considered in patients with a positive epidemiological exposure history and a compatible clinical syndrome, the risk of a patient with EVD arriving in
Ontario is higher than usual as a result of this outbreak. The number of Canadians presently in the affected area is small, making this a low probability event.

EVD should be initially suspected in all patients with fever and a positive travel history or epidemiological exposure within 21 days of illness onset. A positive travel history includes travel to any country where EVD outbreaks are occurring. Check the MOHLTC EVD website at www.ontario.ca/ebola for an updated list of geographic countries/areas currently affected by EVD.

Additionally, EVD (or other VHF) should be suspected in patients with a compatible clinical illness that have travelled within 21 days to any country where sporadic cases of VHF occur, or where Lassa fever is endemic. Clinical assessment of risk of EVD, including risk factors of exposure, clinical status and consideration of differential diagnoses is required prior to requesting testing for Ebola virus.

2. TESTING RECOMMENDATIONS FOR CASES OF SUSPECTED EBOLA VIRUS DISEASE

If a case of EVD is suspected following clinical and epidemiological assessment, the following testing should be performed urgently. 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 EVD. Co-infection with Ebola virus and malaria has been described.

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

- **Testing for Ebola virus** is performed at PHOL Toronto. Prior to submitting samples for testing, please contact the PHOL Customer Service Centre at 416-235-6556 or 1-877-604-4567 (Monday to Friday 7:30 a.m. – 7:00 p.m., Saturday 8:00 a.m. – 3:45 p.m.), or the Duty Officer after-hours at 416-605-3113.

  - 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 sample taken at least 72 hours after symptom onset, no further Ebola testing is needed.

  - If a negative test result is received from a sample that was taken less than 72 hours after symptom onset, a second sample 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.

- Negative results from PHOL are final and can be acted upon.

- Patients at risk for Ebola often develop fevers from other causes (e.g. malaria, typhoid, 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.
• **Examination for malaria** (Testing may include thin smears, immunochromatographic/rapid tests or PCR).
  - Testing for malaria is available at PHOL.
  - For malaria testing to be performed at PHOL, collect a minimum of 2 mls of blood in a lavender top (EDTA) tube.
  - Do not send pre-prepared malaria smears to PHOL.
  - As with samples sent for Ebola virus testing, samples shipped to PHOL for malaria testing collected from suspected or confirmed EVD cases, require handling and shipping according to the international procedures for transport of Category A infectious substances.

• **Other essential testing includes:**
  - Two sets of blood cultures
  - Complete blood count, INR, PTT, electrolytes, creatinine, transaminases, glucose

• **Note:** 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, testing for influenza and other respiratory viruses (as they are non-essential for acute management).
Table 1: Recommended specimen collection guidelines for diagnosis/detection of Ebola virus disease and malaria (if testing for malaria is requested of PHO Laboratories).

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Test</th>
<th>How to submit</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA Blood*‡</td>
<td>Ebolavirus PCR</td>
<td>2 tubes of 2-4 mls each in EDTA containing tubes‡</td>
</tr>
<tr>
<td>EDTA Blood*‡</td>
<td>Malaria rapid test, thin smear and PCR</td>
<td>1 tube of 2-4 mls in EDTA containing tube‡</td>
</tr>
</tbody>
</table>

* Tubes should not be opened/pretreated prior to transport
‡ Ebolavirus PCR and Malaria testing require separate tubes
‡ One tube will be tested by PHOL and the second will be reserved for any testing performed at the National Microbiology Lab (NML)
**Figure 2: Testing Flow for Ebola Virus Disease (EVD) in Ontario**

Prior to sample collection, immediately call:

1. Local/hospital infection prevention and control team, occupational health and safety team, and an infectious diseases specialist

2. Local/hospital laboratory management and/or microbiologist

3. PHO Laboratory Customer Service Centre at 416-235-6556 or 1-877-604-4567 (Monday to Friday 7:30 a.m. – 7:00 p.m., Saturday 8:00 a.m. – 3:45 p.m.), or the Duty Officer after-hours at 416-605-3113.

4. The local Public Health Unit

---

### Ebola testing

Ebola testing is performed at PHOL Toronto.

PHO will send samples to the National Microbiology Laboratory (NML), Winnipeg, for further testing, as required.

Samples are to be shipped directly from the submitting hospital to PHOL Toronto. An interim guidance document on the shipping process of suspect Ebola specimen is available at [http://www.publichealthontario.ca/en/eRepository/EVD_Shipping.pdf](http://www.publichealthontario.ca/en/eRepository/EVD_Shipping.pdf).

### Malaria testing

Testing performed at the local/hospital laboratory using local and approved protocol (if available).

Confirmatory/reference testing performed at the Public Health Ontario Laboratory.

### Other testing

Only specimens essential for diagnosis or monitoring should be obtained and tested at the local/hospital laboratory.

This testing should be performed at the local/hospital laboratory and requires prior consultation with the laboratory director and may include two sets of blood cultures, a complete blood count, INR, PTT, electrolytes, creatinine, transaminases, and glucose.
3. SPECIMEN COLLECTION GUIDELINES

- For guidance on appropriate occupational health & safety and infection prevention & control measures, please see the Chief Medical Officer of Health Directive for Acute Care Settings at www.ontario.ca/ebola.

- Before the collection of specimens to be submitted for Ebola virus testing, contact the PHOL Customer Service Centre at 416-235-6556 or 1-877-604-4567 (Monday to Friday 7:30 a.m. – 7:00 p.m., Saturday 8:00 a.m. – 3:45 p.m.), or the Duty Officer after hours 416-605-3113. The hospital’s infection prevention and control team, occupational health and safety team, laboratory management and microbiologists must also be contacted prior to the collection of any specimens. The following should be observed in the collection of all specimens from patients suspected to have EVD:

  - 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.

  - If labels will withstand cleaning with disinfectant, consider labeling specimen tubes before collection to reduce specimen handling. Labels must bear the patient’s name, hospital identification code, source of the specimen, date of collection, and the nature of the suspected infection.

  - Cleaning the exterior of the specimen container within the patient room is important.

  - Once the specimen is collected, the entire outside surface of each specimen container should be wiped with a hospital-grade disinfectant, then the outer layer of gloves can be removed.

  - Clinical laboratory specimens should each be placed into separate sealable plastic biohazard bags, and then sealed, and the outer surface should be decontaminated with hospital-grade disinfectant.

  - 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.

  - Specimens should then be placed in a durable, leak-proof secondary container ideally held in the antechamber of the patient room, or if not possible, outside of the patient room. Once sealed, the secondary container must be delivered directly to the specimen handling area of the laboratory and should not be left unattended while awaiting transportation.

  - 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 sample receipt until testing is complete.

  - Aliquotting of specimens collected for EVD should be avoided if at all possible.

  - When both Ebola and malaria testing are being requested of the PHO Laboratories, the requests may be combined on one PHO Laboratories requisition. Other tests requested on the same requisition will be cancelled.
• If additional tests are requested of PHOL, separate samples must be submitted, each with its own laboratory requisition, clearly stating patient’s suspected diagnosis and risk factors. Other non-essential microbiology tests will be delayed pending Ebola virus testing results.

4. SHIPPING INSTRUCTIONS


• Specimen should be stored in a refrigerator until being shipped for testing.

• Specimens should be shipped with ice packs.

• **Ship specimens in separate sealable leak-proof biohazard bags** placed in a Transportation of Dangerous Goods Regulations (TDGR) approved shipping container.

• Packing, shipping and transport of all samples related to a suspected case of EVD must comply with the requirement of the TDGR and be performed by a Transportation of Dangerous Goods certified individual. These regulations require handling and shipping patient’s samples according to the international procedures for transport of category A infectious substances (UN2814; http://www.tc.gc.ca/eng/tdg/clear-tofc-211.htm).

• To facilitate purchase of Type 1A containers, a list of vendors can be accessed at: http://www.tc.gc.ca/eng/tdg/moc-infectious-type1a-349.html

• If urgent support is required for packing, shipping and transport of samples related to a suspected case of EVD, please contact the National Microbiology Laboratory (NML) Operations Centre Director (OCD) at 1-866-262-8433.

   For non-urgent general questions about TDGR shipping please contact the Canadian Transport Emergency Centre (CANUTEC) information line at (613) 992-4624.

5. SPECIMEN HANDLING/PROCESSING IN THE LABORATORY

• Any laboratory staff involved in manipulating, processing, or testing of non-inactivated clinical specimens, including malaria smears, should do so in a certified class II biological safety cabinet (BSC) with enhanced precautions including:
  • fluid-resistant, long-sleeved cuffed gown
  • gloves: note double gloves may be used when manipulating uncontained specimens in the class II BSC. Once the procedure in the BSC is complete, the outer gloves (which may have been contaminated) are removed and discarded inside the BSC and the inner gloves are removed and discarded outside the BSC.
  • full face shield
  • fit-tested N95 or other approved particulate respirator

• Laboratory settings are more controlled than clinical settings. There is no uncontrolled exposure to body fluids, and work is done in a closed system or in a BSC II. Therefore the enhanced PPE recommended above is appropriate to protect the health care provider from infection in this environment. Personnel who conduct laboratory testing in clinical care areas are subject to additional precautions outlined in the Acute Care Directive at www.ontario.ca/ebola.
Pre-treatment

- 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 (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.” [http://www.cdc.gov/HAI/pdfs/bbp/VHFinterimGuidance05_19_05.pdf](http://www.cdc.gov/HAI/pdfs/bbp/VHFinterimGuidance05_19_05.pdf), accessed August 11, 2014)

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

- Blood smears (for malaria, thin blood films) are not infectious for EVD 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, must be done wearing full PPE as described above, and any manipulation of the specimen, including the removal of the cap, must be done in a Class II BSC.

- Non-inactivated specimens can be processed for hematologic 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 hematology and chemistry testing are not available, you must discuss testing with the core laboratory director.

- Routine cleaning and disinfecting procedures after use can be used for automated analyzers as recommended by the manufacturer.

- All waste including specimen tubes, cuvettes and other liquid or solid waste must be disposed safely as biohazardous waste.
6. 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](http://www.cdc.gov/vhf/ebola/hcp/interim-guidance-specimen-collection-submission-patients-suspected-infection-ebola.html)
  - Australian Public Health Laboratory Network: [Laboratory Precautions for Samples Collected from Patients with Suspected Viral Haemorrhagic Fevers](http://www.cdc.gov/vhf/ebola/hcp/interim-guidance-specimen-collection-submission-patients-suspected-infection-ebola.html)
- Ministry of Health and Long-Term Care
  - [www.ontario.ca/ebola](http://www.ontario.ca/ebola)
- PHO
  - Ebola Virus Disease (EVD) web page
  - PHO laboratory services and testing information
  - [Viral Haemorrhagic Fever Testing Information Sheet](http://www.ontario.ca/ebola)
  - [PHOL General Test Requisition form](http://www.ontario.ca/ebola) (for malaria testing in suspect EVD cases)
- Biosafety information relevant to viral haemorrhagic fevers
  - PHAC: [Pathogen Safety Data Sheets and Risk Assessment (index)](http://www.ontario.ca/ebola)
  - PHAC: [Canadian Biosafety Standards and Guidelines –First Edition](http://www.ontario.ca/ebola)
  - PHAC: [Interim Biosafety Guidelines for Laboratories Handling Specimens from Patients Under Investigation for Ebola Virus Disease](http://www.ontario.ca/ebola)
  - Transport Canada: [Transportation of Dangerous Goods Regulations](http://www.ontario.ca/ebola)