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

August 14, 2014

This document has been updated as of August 14, 2014, based on the best available evidence at that time. 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 are uncertain.

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. Beginning in March 2014 an EVD outbreak began in West Africa. See the World Health Organization’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 still 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 (e.g. Sierra Leone, Guinea, Liberia, and Nigeria as of August 2014.) Check the World Health Organization [WHO] website at: www.who.int/csr/disease/ebola/en/ for updated list of active outbreaks) or any direct exposure to a human or animal with known or suspected 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.

Current case definitions can be found in the Public Health Agency of Canada (PHAC) website located at: www.phac-aspc.gc.ca/id-mi/vhf-fvh/ebola-professionals-professionnels-eng.php

Note: case definitions are routinely modified in response to a case or outbreak occurring.

2. TESTING RECOMMENDATIONS FOR CASES OF SUSPECTED EBOLA VIRUS DISEASE

If a case of EVD is suspected after clinical 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.

Please discuss with your local laboratory director prior to any sample collection to ensure that any specimens for testing are transported appropriately and testing is performed safely (for sample flow, see Figure 1):

- Testing for Ebola virus (performed at the National Microbiology Laboratory in Winnipeg - contact PHOL Customer Service Centre at 416-235-6556 or 1-877-604-4567 to discuss sample submission) (see Table 1).

  - Please note: Ebola virus is detected in blood only after onset of fever and it may take up to 4 days after that time for the virus to reach detectable levels. If the initial sample is collected less than 4 days after fever onset and ongoing clinical suspicion of disease, a second sample may be required.
- Examination for malaria (screens may include thin and thick smears, or Immunochromatographic tests).
  - Screen testing (if unavailable locally) and confirmation testing for malaria is available at PHOL. Please contact PHOL Customer Service Centre at 416-235-6556 or 1-877-604-4567 to discuss sample submission

- Other testing to consider includes:
  - Two sets of blood cultures
  - Complete blood count, INR, PTT, electrolytes, creatinine, transaminases, glucose
  - Testing that should be avoided includes: cross matching of blood (cannot be performed safely) and cultures of non-sterile sites (non-essential). If transfusion is required, blood type O Rh negative blood (universal donor) should be used.

Table 1: Recommended specimen collection guidelines for diagnosis/detection of Ebola virus disease.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Test</th>
<th>How to submit</th>
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</thead>
<tbody>
<tr>
<td>Blood</td>
<td>PCR &amp; viral culture</td>
<td>2-4 mls in tube containing EDTA</td>
</tr>
<tr>
<td>Blood</td>
<td>Serology</td>
<td>2-4 mls in serum separator tube (SST)</td>
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</tbody>
</table>
Prior to sample collection, immediately call

1. Local/hospital infection prevention and control and infectious diseases specialist

2. Local/hospital laboratory director and microbiologist

3. PHO Laboratory Customer Service Centre at 416-235-6556 or 1-877-604-4567

### Ebola testing

Performed at the National Microbiology Laboratory (NML), Winnipeg, MB.

This is to be shipped directly from the submitting hospital to NML (PHOL will alert NML, and provide contact information).

### Malaria testing

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

Screen (if unavailable locally), or confirmation 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 lab 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

Before the collection of specimens to be submitted to PHO Laboratory and/or NML, contact the PHOL Customer Service Centre at 416-235-6556 or 1-877-604-4567. Local hospital infection prevention and control, laboratory directors and microbiologists must also be contacted prior to the collection of any specimens. The following should be observed in the collection of all patient specimens suspected of 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.

- The same protective clothing as described for other hospital staff should be worn by those obtaining laboratory specimens (refer to Infection Prevention and Control Guidance for Patients With Suspected or Confirmed Ebola Virus Disease (EVD) in Ontario Health Care Settings) with the addition of double gloving to facilitate the decontamination of the exterior of the specimen container. These include:
  - fluid-resistant, long-sleeved cuffed gown
  - double gloves
  - full face shield
  - surgical or procedure mask

- The need for additional personal protective equipment (PPE) such as foot/leg coverings, head coverings, the use of fit tested N95 or a similar mask or specific biohazard suits depends on the potential for fluid contact or aerosolization as determined by the procedure being performed and the presence of clinical symptoms that increase the likelihood of contact with body fluids. It should be noted that these instances will be rare and the PPE recommended above is sufficient to protect the health care provider from infection.

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

- 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.

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

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

- 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 hospital-grade disinfectant wipe or disinfectant before leaving the patient's room.
• Specimens should then be placed in a **durable, leak-proof secondary container prior to** direct delivery to the specimen handling area of the laboratory.

• **Automated delivery (pneumatic tube) systems should not be used** as they may disseminate aerosols in the event of a spill or breakage.

• Samples should not be left unattended while awaiting transportation.

• **Laboratory staff should be alerted to the nature of the specimens**, which once received should remain in the custody of designated persons from the sample receipt until testing is complete.

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

• Each sample for EVD-specific testing must be submitted with its own separate requisition form requesting Ebola virus testing from NML (the NML special pathogens requisition can be accessed at [https://www.nml-lnm.gc.ca/guide2/files/26-Requisition-form-Special%20Pathogens-ENG.pdf](https://www.nml-lnm.gc.ca/guide2/files/26-Requisition-form-Special%20Pathogens-ENG.pdf)). Other tests requested on same requisition will be cancelled.

• If additional tests are requested, separate samples must be submitted, each with its own laboratory requisition, **clearly stating patient’s suspected diagnosis and risk factors**. Other tests may be delayed pending Ebola virus testing results.

• Malaria testing requested from PHOL must be accompanied by the **PHOL General Test Requisition form** with the same information.

### 4. SHIPPING INSTRUCTIONS

• Packing, shipping and transport of all samples related to a suspected case of EVD must comply with the requirement of the Transportation of Dangerous Goods (TDGR) and be performed 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; [http://www.iecanada.com/ienow/2010/oct_10/Protocol_Infectious_Substances.pdf](http://www.iecanada.com/ienow/2010/oct_10/Protocol_Infectious_Substances.pdf); [http://www.tc.gc.ca/eng/tdg/clear-tofc-211.htm](http://www.tc.gc.ca/eng/tdg/clear-tofc-211.htm)).

• 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) (1-866-262-8433).

• For non-urgent questions about shipping please contact the CANUTEC information line at (613) 992-4624.

• Store samples in refrigerator or in freezer (if expected to be greater than 24 hours) until being shipped for testing.

• Refrigerated samples should be shipped with ice packs, and frozen specimens should be shipped on dry ice.

• **Ship specimens in separate sealable leak-proof biohazard bags** placed in a TDGR approved shipping container.
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 class II biological safety cabinet with enhanced precautions, including:
  - fluid-resistant, long-sleeved cuffed gown
  - gloves
  - full face shield
  - fit-tested N95 or other approved particulate respirator

- The need for additional PPE such as the use of foot/leg coverings, head coverings or specific biohazard suits depends on the potential for fluid contact as determined by the procedure being performed and the presence of clinical symptoms that increase the likelihood of contact with body fluids. It should be noted that these instances will be rare and the PPE recommended above is sufficient to protect the health care provider from infection.

- The use of N95 or other approved particulate respirator is recommended for laboratory testing due to the possibility of aerosol generating procedures in the opening, processing and testing in the laboratory setting, despite the lack of evidence of transmission in this manner.

Pre-treatment

- All pretreatment and manipulation should occur within a class II biological safety cabinet 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.” And later in the text “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)

- Pre-treatment 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

- Non-inactivated specimens can be processed in automated analysers for hematologic and biochemical testing that are closed 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 before any specimen collection. If approved, all specimen handling from the accessioning window through to the running on the machine 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 the BSL II cabinet.

- 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.

- See Australian Public Health Laboratory Network: Laboratory Precautions for Samples Collected from Patients with Suspected Viral Haemorrhagic Fevers for further information on methods for viral inactivation and how to conduct non-microbiological essential laboratory testing.

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)

- PHO resources
  - [Ebola Virus Disease (EVD) web page](http://www.cdc.gov/vhf/ebola/hcp/interim-guidance-specimen-collection-submission-patients-suspected-infection-ebola.html)
  - [PHO laboratory services and testing information](http://www.cdc.gov/vhf/ebola/hcp/interim-guidance-specimen-collection-submission-patients-suspected-infection-ebola.html)
  - [Viral Haemorrhagic Fever Testing Information Sheet](http://www.cdc.gov/vhf/ebola/hcp/interim-guidance-specimen-collection-submission-patients-suspected-infection-ebola.html)
  - [PHOL General Test Requisition form](http://www.cdc.gov/vhf/ebola/hcp/interim-guidance-specimen-collection-submission-patients-suspected-infection-ebola.html) (for malaria testing in suspect EVD cases)
• Biosafety information relevant to viral haemorrhagic fevers
  • PHAC: Pathogen Safety Data Sheets and Risk Assessment (index)
  • PHAC: Canadian Biosafety Standards and Guidelines –First Edition
  • Transport Canada: Transportation of Dangerous Goods Regulations