NML

Laboratory testing for Orthoebolaviruses

2026 July

Scope

This document provides practical considerations for public health authorities in Canada on laboratory testing for orthoebolavirus in suspected human cases.

The recommendations contained in this document should be read in conjunction with relevant federal, provincial, territorial and local legislation, regulations, and policies. Recommended measures should not be regarded as rigid standards, but principles and recommendations to inform the development of guidance and harmonize the testing process between provincial laboratories.

While the NML maintains specialized reference and diagnostic services for these HCPs vis-a-vis high containment, distribution of well-characterized, robust assays to be utilized at provincial node laboratories to reliably test high-risk patients greatly increases Canada's ability to detect and treat in a timely manner, in the interest of the patient, public health and public safety. One molecular option includes the BioFire® multiplexed assay test pouches enabling rapid detection of other rare infectious agents within Canada, and facilitating a timely local decision making, which is an important consideration for travelers returning to Canada.

Recommendations for Diagnostic Testing

- **Test for infection by viral detection using RT-PCR on EDTA whole blood.**
  - ***Preferred specimen is whole blood*** (collect in EDTA or citrate tubes), minimum 0.5 ml.
  - Other specimens accepted: serum, CSF, other bodily fluids, swabs, tissues.
  - If multiple specimen types are collected, RT-PCR on whole blood will be prioritized.
  - Due to potential quality control concerns, submission of extracted RNA for testing is not recommended.
  - Procedure as outlined in NML Guide to Services https://public.cnphi-rcrsp.ca/gts/reference-diagnostic-test/15302?labId=1021
- RT-PCR testing of asymptomatic travellers is not recommended.
- RT-PCR testing can be done by provincial public health labs or NML if a jurisdiction does not have RT-PCR capability for orthoebolaviruses.
  - Specimens that are tested at provincial public health labs should also be sent to NML for parallel testing, species identification or differentiation and to satisfy International Health Regulations (IHR) reporting requirements.

- In Canada, BioFire® has two available panels that detect orthoebolaviruses. The Global Fever (GF) Panel (*Research Use Only, RUO*) and Warrior-CA Panel (*in vitro* Diagnostic use, IVD) are closed-systems with a disposable pouch that stores all the necessary reagents for nucleic acid extraction, reverse transcription, polymerase chain reaction (PCR), and detection in order to isolate, amplify, and detect nucleic acid from multiple pathogens within a single whole blood specimen (1, 2).
- Clinical specimens from patients under investigation for high-consequence pathogens (HCPs) may be tested using PCR within a containment level 2 (CL2) laboratory, provided that a certified biological safety cabinet and enhanced CL2 biosafety practices are used, in accordance with PHAC's Canadian Biosafety Standard and Canadian Biosafety Handbook (third edition) (3), and the Biosafety guidelines for laboratories handling specimens from patients under investigation for Ebola disease (4) and the biosafety and biosecurity considerations below.
  - Samples for PCR should be inactivated and extracted within a biological safety cabinet (BSC) and must be inactivated with a guanidine thiocyanate–based extraction buffer with an additional 100% ethanol treatment step included in the extraction protocol to further ensure viral inactivation prior to any handling outside the biological safety cabinet (6, 7, 8).
  - For those using BioFire®, following the manufacturer's instructions and within a BSC, transfer the blood specimen to the BioFire® sample injection vial (containing a guanidine salt-based buffer).
    - Discard the transfer pipette to the biohazard waste stream within the BSC, treat it with appropriate disinfectant within the BSC, or per biohazard waste procedure as part of the local risk assessment.
    - Take care in handling, placing, and pushing the cannula into the pouch to avoid sharps injury and to avoid dripping/leaks from the cannula.
    - After pouch preparation within the BSC and prior to its removal, decontaminate the pouch by wrapping in absorbent soaked in freshly prepared 0.5% sodium hypochlorite for ≥ 10 minutes or other appropriate decontamination procedure, before loading on the FilmArray instrument.
  - Outer gloves should be removed prior to withdrawal from the BSC or per local risk assessment.
- A 'not detected' result does not preclude the presence of an HCP, including viral hemorrhagic fever (VHF) causing viruses not targeted within the PCR assay or BioFire® panel.
- Results must be interpreted in the context of the clinical and epidemiological information available. Concurrent testing at the NML enables an iterative testing approach and enables International Health Regulations reporting for positive cases[1].

- **Repeat testing should be considered when initial nucleic acid amplification test results are negative but clinical suspicion remains high**. A negative result is not a definitive indication that infection will not occur during the incubation period of the virus.
  - Available evidence suggests that viral RNA for Ebola virus and related orthoebolaviruses may be undetectable by RT-PCR very early in the course of infection, particularly around the time of symptom onset and in the first days of illness (5). To mitigate the risk of false-negative results in patients with a compatible exposure history and early or evolving symptoms, a repeat specimen should be collected and tested 24–72 hours after the initial negative sample, but no sooner than 72 hours after the onset of symptoms, guided by clinical assessment and public health risk evaluation.

[1] A multicentre verification using the BioFire® FilmArray® system and the BioFire® GF Panel was completed with CLRN partners in coordination with the NML. Evaluation was completed for Ebola virus as well as the related viruses - Sudan virus, Reston virus, and Tai Forest virus. Bundibugyo virus was not included in this multicentre verification, where gamma irradiated virus stocks were used as materials for detection.
NML performed a preliminary evaluation of the BioFire® GF Panel in containment level 4, testing contrived samples made of human blood spiked with various concentrations of live orthoebolaviruses (including Ebola, Sudan, and Bundibugyo viruses). The BioFire® GF Panel detected these viruses, and performance was comparable to Special Pathogens (SP) lab developed tests (LDTs) for these same viruses.
NML also performed preliminary testing using BioFire® GF Panel and Warrior-CA panels with gamma irradiated Bundibugyo virus; both panels were able to detect this virus.
BioFire Panels available in Canada: Research Use Only Global Fever Panels are marked 'not for diagnostic use'. The panels can be used as a tool to triage and inform testing needs. The Warrior-CA panels are Health Canada approved for diagnostic use.

- Serology testing is not recommended for acute diagnosis of orthoebolaviruses. Serology may be considered only in very limited circumstances to investigate possible past exposure and must be arranged in consultation with SP Diagnostics staff at NML. If serology is performed, all serology samples to be submitted to NML to perform testing:
  - Serum or plasma (minimum 0.5ml) can be tested.
  - Collect serum in red top or serum separator tubes, centrifugation should be avoided.
  - Procedure as outlined in NML Guide to Services https://public.cnphi-rcrsp.ca/gts/reference-diagnostic-test/15302?labId=1021

- For repatriated individuals required to quarantine, testing for orthoebolaviruses should be performed only for symptomatic individuals and is not indicated for baseline screening or serology. Arrangements for testing eligible symptomatic individuals must be made through the Local Public Health Authority and the Provincial Public Health Laboratory, which will liaise with NML and provide advance notice regarding the expected number and types of specimens requiring testing.

SHIPPING

- **Laboratory specimens for orthoebolavirus testing, including serology, must be shipped as Category A** (UN2814 – Infectious Substances, affecting Humans) following the Transportation of Dangerous Goods Regulations (TDGR) and Transport Canada's Shipping Infectious Substances guidance. Specimens suspected of orthoebolavirus should be packaged separately from other specimens and labs should be notified in advance.

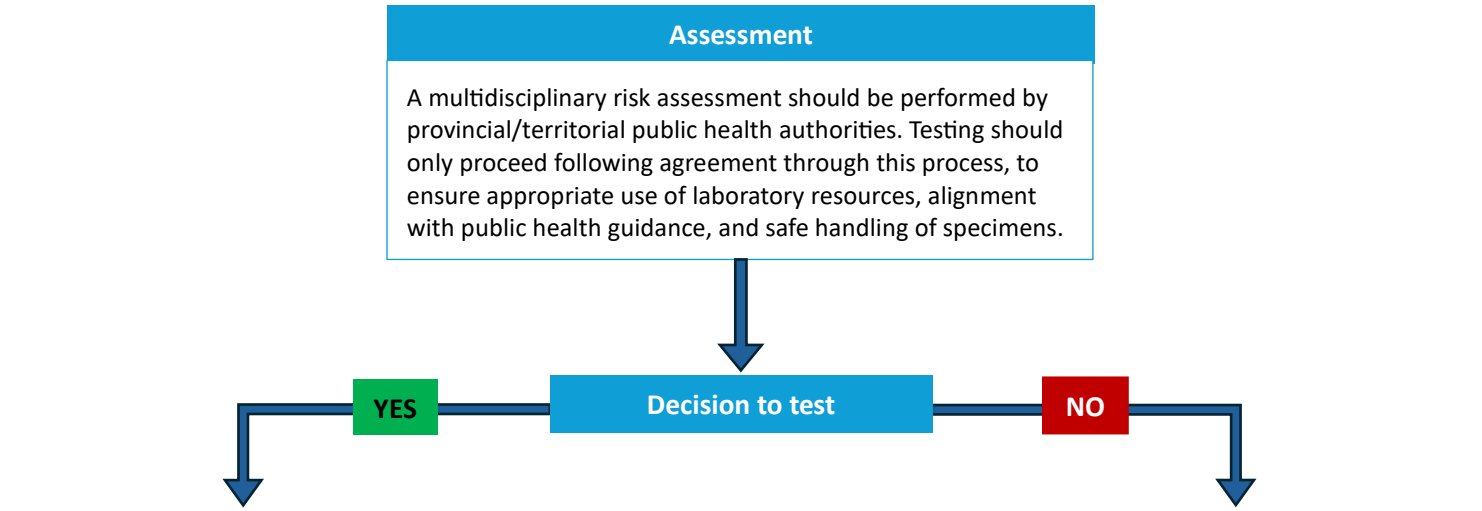
- **PHAC Emergency Response Action Plan (ERAP) activation is required for this shipment.**
  - Labs must communicate with their public health lab to ensure the requirements for the *PHAC ERAP for UN2814 Infectious Substances Affecting Humans, Risk Group 4* are met.
  - Contact **NML Operations Center Director at 1-866-262-8433** to initiate the ERAP as soon as possible in advance of shipping specimens by any means of transport and to any location.

Recommendations for Responding to Routine Health Events for Individuals in Quarantine

- Samples from persons who are under the federal Quarantine Act, but do not meet the clinical and epidemiological criteria for Ebola disease Person Under Investigation, following a local clinical and epidemiological assessment may be tested for other health conditions and should be treated with regular precautions according to their other medical conditions and risks.

- A multidisciplinary risk assessment should be performed by provincial/territorial public health authorities. Testing should only proceed following discussion through this process, to ensure appropriate use of laboratory resources, alignment with public health guidance, and safe handling of specimens.

Testing strategy for Orthoebolaviruses



Test for Orthoebolaviruses

Action:
Perform **RT-PCR testing** on whole blood (EDTA preferred or serum/plasma)

BioFire® Panel testing or alternative molecular assays if available. Samples can also be submitted to NML directly.

Samples that are tested at provincial public health labs should also be sent to NML for parallel testing to satisfy International Health Regulations (IHR) reporting requirements.

***Testing bodily fluid samples other than blood has generally lower diagnostic value [5]; blood will be prioritized, if other sample types are submitted in parallel (i.e. swabs) they may be tested IF the blood is positive

If the sample is positive - whole genome sequencing can be considered to support the epidemiological interpretation of the outbreak. It is not required for case management.

Interpretation:

- RT-PCR positive → manage as a confirmed case.

- RT-PCR negative and symptoms persist:
  - If the initial orthoebolavirus RT-PCR test is negative, repeat testing should be considered over the next 24–72 hours, as testing very early after symptom onset may yield false-negative results.
  - Perform immediate RT-PCR testing in case of any clinical deterioration.
  - **If any of the RT-PCR tests are positive → classify and manage as a confirmed case**

No testing for Orthoebolaviruses

Clinical evaluation to rule out alternative diagnoses and testing for other health conditions, if required.

Reassessment if required

As additional information emerges (e.g. change in the patient's clinical status or new epidemiologic risk factors), the initial assessment should be revisited, and testing decisions re-evaluated.

Testing Safety and biosecurity considerations

- All clinical specimens from patients under investigation for orthoebolavirus infection should be treated as potentially infectious and managed per PHAC's Biosafety guidelines for laboratories handling specimens from patients under investigation for Ebola disease (4) and the Canadian Biosafety Standard laboratory biosafety guidelines (3).
- Engineering and administrative controls, PPE, and waste management should be defined with a local risk assessment. Personnel should be limited in the areas where clinical specimens from patients under investigation for orthoebolavirus infection are handled or stored.
- At minimum, clinical specimens must be handled following requirements for Containment Level 2 (CL2) per the Biosafety guidelines for laboratories handling specimens from patients under investigation for Ebola disease within a primary containment device, such as certified Class II biosafety cabinet (BSC) prior to inactivation and with additional operational practices specified in the Guideline.
- Routinely disinfect all surfaces, including of all items, before removal from a BSC and items outside the BSC in closed systems such as blood culture bottles before loading, with suitable disinfectant and appropriate contact times such as those listed in the Guidelines and the Pathogen Data Safety Sheet.
- Activities with the potential to create infectious aerosols, such as pipetting, should be performed in a certified BSC or other primary containment device. Sealed safety cups or sealed rotors should be used and opened and unloaded in the BSC.
- Hand hygiene should be practiced regularly and after the removal of PPE.
- All staff should be trained and competent in biosafety and incident/exposure response.
- Caution should be practiced with blood cultures; these should not be handled outside a closed system before the orthoebolavirus result is determined or if the result is positive.
- Routine clinical testing (e.g. CBC, chemistries, blood gases, urine, CSF) may continue for patients under investigation for or with confirmed Ebola virus disease when required for clinical management, in accordance with institutional policies and the Biosafety guidelines for laboratories handling specimens from patients under investigation for Ebola disease.
- Handling, propagating, and storing orthoebolaviruses (i.e., cultivated or intentionally collected or extracted virus) is regulated under the *Human Pathogens and Toxins Act* and is only permitted in a Containment Level 4 facility operating under a pathogen and toxin licence issued by the Public Health Agency of Canada (Human Pathogens and Toxins Act).

Summary

- NML will support P/T testing for individuals associated with the on-going orthoebolavirus response stemming from the DRC Bundibugyo virus outbreak.
  - Testing should only be done on persons under investigations.
  - Blood specimens (collected in EDTA tubes) are the preferred specimen for analysis.
  - Lab-developed tests and reference testing are available at the NML.
  - Repeat testing should be considered if initial results are negative.

For specimen submission or laboratory consultation please contact:

NML Special Pathogens Program nml.sp-lnm.asp@phac-aspc.gc.ca

24/7 - NML Operations Center Director at 1-866-262-8433

This document was developed in collaboration with members of the Canadian Public Health Laboratory Network (CPHLN) Council.

References:

1. FilmArray® Global Fever Panel – RUO Instructions for Use.
2. FilmArray® NGDS Warrior Panel – CA Instructions for Use.
3. Canadian Biosafety Standard, Third Edition - Canada.ca
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5. Clinical management of patients with viral haemorrhagic fever: a pocket guide for front -line health workers: interim emergency guidance for country adaptation. World Health Organization. ISBN 978 92 4 154960 8(NLM classification: WC 534).
6. Smither SJ, Weller SA, Phelps A, Eastaugh L, Ngugi S, O'Brien LM, Steward J, Lonsdale SG, Lever MS. Buffer AVL Alone Does Not Inactivate Ebola Virus in a Representative Clinical Sample Type. *J Clin Microbiol*. 2015 Oct;53(10):3148-54. doi: 10.1128/JCM.01449-15. Epub 2015 Jul 15. PMID: 26179307; PMCID: PMC4572529.
7. Haddock E, Feldmann F, Feldmann H. Effective Chemical Inactivation of Ebola Virus. *Emerg Infect Dis*. 2016;22(7):1292-1294. https://doi.org/10.3201/eid2207.160233
8. Cutts T, Leung A, Banadyga L, Krishnan J. Inactivation Validation of Ebola, Marburg, and Lassa Viruses in AVL and Ethanol-Treated Viral Cultures. *Viruses*. 2024 Aug 24;16(9):1354. doi: 10.3390/v16091354. PMID: 39339831; PMCID: PMC11436171.