Public Health Ontario | Santé publique Ontario

LABORATORY GUIDANCE

Diagnostic Testing for Viruses That Cause Hemorrhagic Fevers

Updated: May 2026

Introduction

This document provides an overview of the testing process for risk group 4 (RG4) viruses that cause hemorrhagic fever syndromes (VHFs), including Ebola disease (ED), at PHO. It replaces the previously published documents: Laboratory Guidance for Viral Haemorrhagic Fevers, including Ebola Virus Disease, and Laboratory Guidance for Specimens Requiring Emergency Response Assistance Plan for Transport Within Canada. Current nomenclature is used here to describe these viruses and the diseases they cause .[1,2]

This document provides specific information on:

- Testing methods
- Specimen requirements for testing
- Preparation of shipment and processing of specimens for VHF Testing
- Result interpretation and confirmatory testing

Scope

This document **does not** provide guidance or recommendations for:

- Performing clinical symptom and exposure risk assessments or measures related to infection prevention and control for RG4 viral pathogens. Refer to Public Health Ontario's VHF Webpage for the relevant information.[1-2]
- Testing for other non-RG4 VHF-causing viruses not mentioned in this document (e.g. dengue virus, yellow fever virus, among others). Information on diagnostic testing for these viral pathogens can be found on Public Health Ontario's Laboratory Test Information Index.[3]

Microbiology Laboratory Testing in the Setting of a Suspected VHF

Testing for RG4 viruses that cause VHFs is not performed routinely. If you suspect a patient has a VHF, consult the Ontario Ministry of Health **Notification Pathway for Special Pathogens** for a description of the provincial VHF coordination algorithm and Public Health Ontario's VHF Symptom and Exposure Risk Assessment form for information required for the clinical evaluation.[1,4]

Key Considerations When Testing for Viruses That Cause VHFs:

- VHF test requests require planning, notification and communication amongst all relevant stakeholders, including PHO, to ensure the safety of all parties involved.
- The risk of a VHF should be evaluated in the context of the patient's clinical signs/symptoms, including onset date, with consideration given to relevant travel (including specific locations visited) and exposure history when determining the differential diagnosis. This information is required to support the request.
- The clinical teams must ensure that any requests for VHF testing are appropriate and in keeping with a suspected VHF. Other, more common and potentially fatal infectious diseases including malaria, typhoid fever, and/or bacteremia, should also be considered in individuals with a compatible illness, as appropriate.

Methods to Detect VHF-Causing Viruses at PHO

Real-time polymerase chain reaction (RT-PCR) assays are the preferred method to detect VHF-causing viruses. These assays can detect viral nucleic acids in clinical specimens collected from individuals suspected of a VHF.[5-7] PHO offers both commercial (BioFire FilmArray) and laboratory-developed RT-PCR tests for several RG4 viruses that cause VHFs (Table 1).

Table 1: RT-PCR Tests for RG4 VHF-Causing Viruses[a] That Are Available at PHO

Orthoebolaviruses	Orthomarburgviruses	Other
Bundibugyo virus	Marburg virus	Crimean-Congo hemorrhagic fever virus
Ebola (Zaire) virus	-	Lassa virus
Sudan virus	-	Rift valley fever virus

[a] Testing for other RG4 viruses not listed in Table 1 may be available upon request.

The BioFire multiplex RT-PCR assay was validated at PHO in collaboration with the NML and other laboratory partners across the Canadian Laboratory Response Network (CLRN). The laboratory-developed RT-PCR assays validated at PHO were initially developed and validated by the NML. Both BioFire and laboratory-developed assays may detect multiple gene targets for one of the viruses listed in Table 1.[8,10,12] Where possible, based on genome availability, PHO's laboratory reviews RT-PCR primer sequences against viral genomes deposited in publicly available databases to verify whether VHF viral variants associated with ongoing outbreaks are detectable by the RT-PCR assays. Any test limitations will be communicated by the PHO Microbiologist during the health system partner coordination calls.

Testing for Other Pathogens at PHO in Suspected VHF Cases

Other more common and potentially fatal infectious diseases (e.g. malaria, typhoid fever and bacteremia) should be considered in the differential diagnosis of suspected VHF cases.

Malaria

Malaria testing is available at PHO's laboratory and can be performed on specimens from suspect VHF cases, upon request. The request must be specified on the General Test Requisition when submitting specimens for VHF testing or testing will not be performed.

Do not send pre-prepared malaria smears/slides to PHO's laboratory due to biosafety risks.

Other Differential Agents of Febrile Illness in Travelers

The BioFire FilmArray multiplex PCR assay may also detect other co-pathogens, such as West Nile virus, dengue virus, yellow fever virus, chikungunya virus, Zika virus, typhoid fever, paratyphoid fever, leptospirosis, tularemia, plague, anthrax, malaria, and leishmaniasis. However, depending on the agent and the stage of illness, RT-PCR results (including BioFire results) may not always rule out infection with these other pathogens. If detected, BioFire results must be inteterpreted in the context of the clinical and exposure history, and additional testing may be warranted for these pathogens if clinically indicated once the risk of RG4 VHF infection is no longer present.

Other Infectious Agents

All other non-essential infectious disease testing should be avoided until RG4 viruses causing VHFs are excluded, if there is a high index of suspicion. No additional microbiological testing will be performed at PHO until the RG4 VHF-causing viruses under investigation have been ruled out. This includes respiratory virus testing, tests that require culture and any other non-viral tests that are non-essential for acute patient management.

Routine blood cultures (e.g. for bacterial analysis) may be warranted for the acutely febrile patient but are not performed by PHO. Consult the hospital/community laboratory that provides microbiology services to your institution who may perform that testing in this setting and can provide additional guidance in accordance with institutional policies.

Requirements for VHF Testing at PHO

Activities Prior to Specimen Collection

Prior to collecting specimens for VHF testing:

- If the primary or other health sector organization suspects that a patient's symptoms are compatible with a RG4 VHF, consult the Ministry of Health **Notification Pathway for Special Pathogens** for details regarding the notification process. Where warranted, they may facilitate coordination of the provincial and federal health system partners, including PHO, to respond to the situation.
- The case must also be discussed with your local laboratory management team for awareness and to ensure that, if the decision to proceed with RG4 VHF testing is made at the health system partners coordination call, any specimens are collected and transported in accordance with the Transportation of Dangerous Goods Act/Regulations, and any non-VHF testing is performed safely in accordance with a local risk assessment.[19]
- Due to the biosafety concerns associated with the viruses described in this document, specimens for RG4 VHF testing should not be collected prior to the provincial coordination call to discuss the case. If the decision to proceed with testing has been made at the provincial health system partners coordination call, it is recommended to notify the local/regional microbiology laboratory site that provides routine service to your institution and PHO's laboratory, if applicable, if any specimens were recently collected and tested or were collected and are already in transit for microbiology testing prior to considering a RG4 VHF on the clinical differential diagnosis/proceeding with RG4 VHF testing.

Specimens to Collect and Submit for VHF Testing

The following information will assist with specimen collection and submission if the clinical team decides to proceed with RG4 VHF testing after consulting with provincial partners.

Collect only specimens listed in Table 2 for PHO submission. Staff experienced in the required techniques and safety procedures, including proper use of personal protective equipment (PPE), should collect specimens. Refer to PHO's Infection Prevention and Control Management of Viral Hemorrhagic Fever in Acute Care and your institutional guidance for additional information.

Table 2: Specimen Requirements for RG4 VHF Viruses and Malaria Testing

Pathogens of Interest	Tests Performed at PHO	Specimen Information[a,b,c]
RG4 viruses causing VHF:[13] • Bundibugyo virus • Crimean-Congo Hemorrhagic Fever virus • Ebola virus • Lassa virus • Marburg virus • Sudan virus	RT-PCR (including BioFire and laboratory-developed tests)	**Requirements:** • 2 x EDTA-Blood tubes specifically for VHF testing • One tube will be tested by RT-PCR at PHO • One tube will be forwarded to the NML **If the patient is an ADULT:** • 2 to 4 mL of whole blood is required per tube **For PEDIATRIC patients or if collection is difficult:** • 0.5 to 1 mL of whole blood is required per tube
Malaria	Malaria rapid test Thin smear Real-time PCR	**Requirements:** • 1 x EDTA-blood tube specifically for malaria testing • 2 to 4 mL whole blood is required in the tube

[a] Tubes should **not** be opened or pretreated prior to transport to PHO.
[b] **DO NOT** submit pre-made thick and thin smear slides on patients under investigation for a VHF.
[c] **DO NOT** use glass specimen collection devices/containers, unless there is no other alternative.

Summary of Specimens to be Sent to PHO:

- If BOTH VHF and malaria testing is required: Submit 3 x EDTA-blood tubes to PHO as above
- If ONLY VHF testing is required: Submit 2 x EDTA-blood tubes to PHO as above

Notes: Whole blood is the preferred specimen type for VHF testing.[21] The clinical performance of other specimen types is uncertain.

Each specimen submitted to PHO listed in Table 2 should be labelled with a minimum of two patient identifiers and be accompanied by its own separate PHO laboratory General Test Requisition. Only the tests that have been agreed to should be listed along with the patient's suspected diagnosis and risk factors should be clearly stated. Specimens with non-VHF or non-essential microbiology tests requested will be cancelled.[14] Refer to PHO's Test Information Index for additional information.

Timing of Specimen Collection for VHF Testing

Key considerations regarding the timing of specimen collection:

- Specimens should be collected as soon as possible after symptom onset.[17] Viral nucleic acids may only reach levels that can be detected by RT-PCR 72 hours after symptom onset .[7,8,21]
- The timing of specimen collection for VHF-causing viruses is important for result interpretation. Refer to Table 3 for additional information.
- Testing to discharge hospitalized confirmed cases may be considered, but should follow institutional policies.[17,21]

Table 3: Limitations of VHF Testing Based on Timing of Specimen Collection

Specimen Type	Timing of Specimen Collection from Symptom Onset	VHF Virus RT-PCR Result	Follow-Up Testing Recommendations
Whole Blood	Less than 72 hours	Not Detected	• This does not rule out VHF infection in isolation and requires correlation with clinical, epidemiological and other relevant information. • A second collection for repeat RT-PCR testing is recommended if a VHF is still suspected based on a re-assessment of the patient's clinical condition 24-72 hours later (e.g. no clinical improvement). • The second specimen should be collected at least 72 hours after symptom onset.[17] Refer to Table 2.
Whole Blood	At least 72 hours or more	Not Detected	• No further VHF testing may be needed (once results have been confirmed) if the 21-day incubation period has elapsed. • Re-consider a VHF if the patient is still within the incubation period and develops a new illness consistent with a VHF within 21 days of the last potential exposure • Other pathogens should be investigated if an infectious cause is still suspected after the decision has been made to step down on the VHF investigation

Preparation of Shipment and Processing of Specimens for VHF Testing

Shipping Specimens to PHO for VHF Testing

To facilitate the transfer of specimens collected for RG4 VHF testing to PHO, specialized training/certification, packaging, transportation and documentation is required between hospital sites and laboratories[15,18-19]

Key Considerations When Shipping Specimens for VHF Testing:

- Specimens collected from suspected or confirmed RG4 VHF cases are subject to Part 7 of the Transport Canada Transportation of Dangerous Goods (TDG) regulation, require an Emergency Response Assistance Plan (ERAP), and special shipping and handling.[19,16]
- As defined by Transport Canada an ERAP is a plan that describes the process that is to be followed if the release or the anticipated release of high-risk dangerous goods occurs while they are in transport. These events require special expertise and response equipment.[18]
- All clinical specimens suspected of containing VHF-causing viruses listed in this document require Category A packaging for transportation and must be always shipped with an ERAP.[16, 18-19]
- It is the requesting laboratory/submitter's responsibility to ensure that staff handling specimens are certified in the Transportation of Dangerous Goods (TDG). It is also the responsibility of the submitting laboratory to arrange for a courier that is certified to transport ERAP agents.
- Consult the laboratory that provides routine microbiology services to your institution for additional information regarding internal policies on how to transport specimens to their laboratory prior to sending to PHO, if needed. Additional biosafety guidance is available via PHAC.[16]
- Only ship the specimens outlined in Table 2 in the package that is sent to PHO. Do not include additional specimens for other tests not identified in Table 2 or specimens from other patients in the package. Specific information on the number of specimens contained within the package and the specimen volumes (e.g. amount of blood per tube) will be required for communications during the ERAP process.
- Category A packaging and materials will not be returned to the submitter.

Once the test request has been confirmed, a designated PHO laboratory member will reach out to the submitter of the package requiring ERAP, if necessary. At this time, the PHO laboratory member will request submitter contact information, package information and information on logistics arrangements that will aid in initiating the transportation with ERAP and any subsequent communications with NML and the Ministry of Health Emergency Operations Centre.

The following information will be required by PHO's laboratory for any packages shipped to PHO (or directly to the NML) with an ERAP:

- Name of a member of the patient's clinical management team or designate
- Name of TDG-certified person responsible for preparation of the package containing high risk virus specimen
- Name of courier used for ERAP shipping and the tracking information
- Specimen container volume and number of collection container.

The ERAP number will be provided to the submitter once received. Only one ERAP activation is required if multiple packages are being transported at the same time. The ERAP is activated when the first shipment/package is picked up and is de-activated when the last shipment/package is delivered.

Refer to the NML's Transport Flowchart for Risk Group 4 Pathogen(s) for a general summary of the ERAP initiation process.[20] Additional information on ERAP that is beyond the scope of the current document is provided by Transport Canada.[18]

Processing of Specimens for VHF Testing at PHO

Specimens received by PHO for VHF testing are handled and processed independently from other routine microbiological tests in accordance with PHO's internal risk assessment. PHO's laboratory will perform RT-PCR testing as soon as possible after specimens are received at PHO.

In accordance with federal guidelines, further confirmatory testing by the NML is required for the tests listed in Table 1. The PHO laboratory will facilitate the safe and timely transfer of specimens for VHF testing to the NML in parallel, without waiting for the PHO test results to be released. The turnaround time and reporting plans will be communicated at the time of sample submission.

For guidance on routine specimen handling and processing in laboratories outside of PHO that can be used to inform a local risk assessment, refer to the Public Health Agency of Canada's (PHAC) Biosafety Guidelines for Laboratories Handling Specimens from Patients under Investigation for EVD.[15] Additional information is available from PHAC's Pathogen Safety Data Sheets.

Result Interpretation

Interpretation of VHF RT-PCR Results

Laboratory results for VHF RT-PCR tests performed at PHO will be released to the requesting clinician as soon as they are available. At this time, the appropriate public health unit will also be notified. The reporting plan, including test turnaround times or additional testing required at NML, will be communicated to stakeholders at the time of specimen receipt.

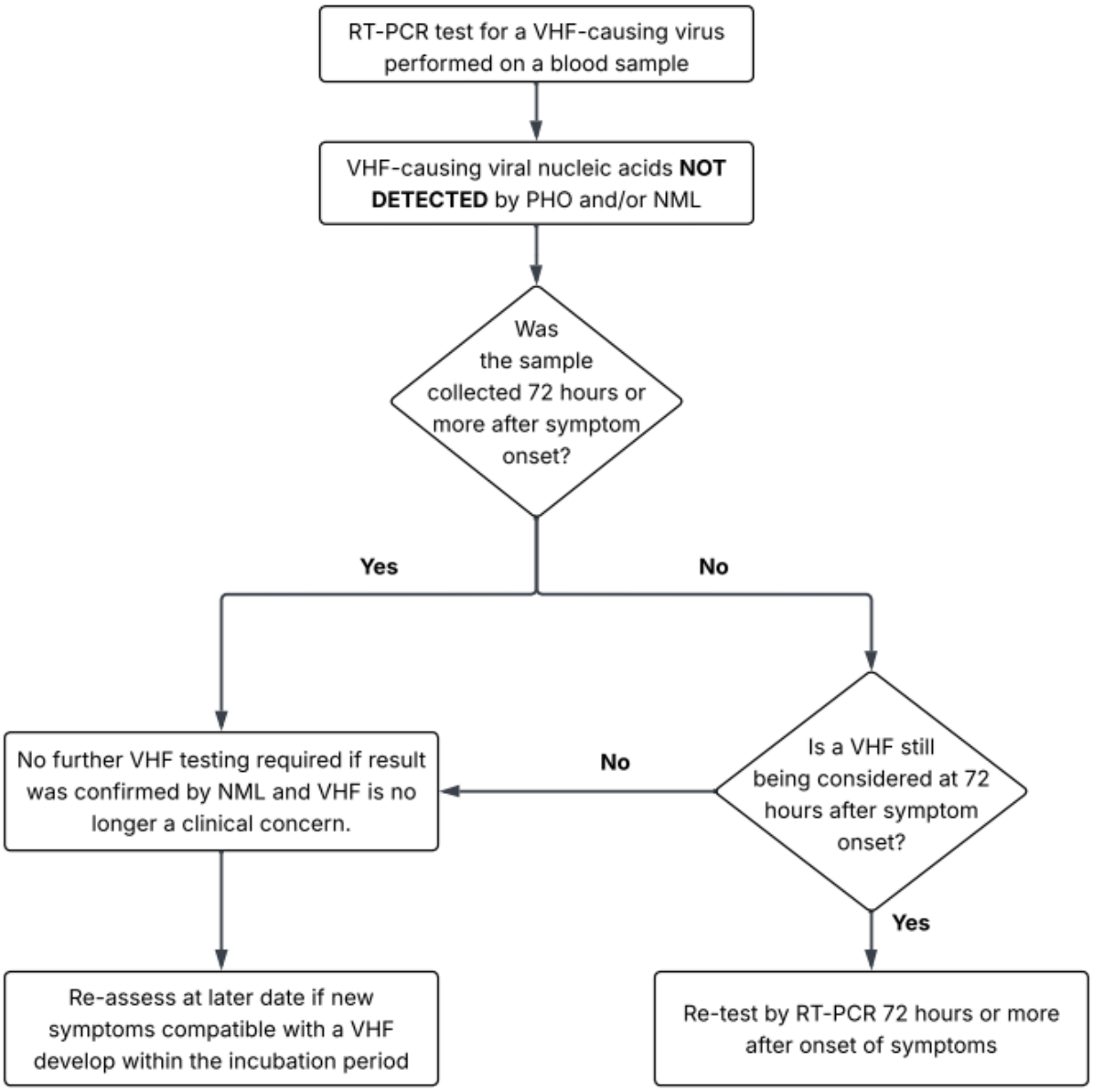
All VHF test results should be interpreted with caution and in the appropriate clinical and epidemiological context (Table 4). Repeat testing may be warranted in some scenarios based on the timing of specimen collection. Refer to Table 4 and Figure 1 for more information.

Table 4: Interpretation of RT-PCR Results for VHF-Causing Viruses at PHO

PHO VHF PCR Result[a,b,c]	Interpretation of PHO Result	Follow-Up Actions
Not Detected	• Nucleic acids from the virus tested were not detected from the specimen. • This result on its own does not exclude infection. • Confirmatory testing will be performed at the NML.	• No additional testing is required if specimen collected >72 hours after symptom onset and the result was confirmed by the NML if there is no longer clinical concern for a VHF. Other testing appropriate for patient care may proceed. • Additional testing is recommended only if viral nucleic acids were not detected in the first specimen and it was collected <72 hours after symptom onset AND a VHF is still suspected after clinical re-assessment of the patient (e.g. no clinical improvement >72 hours after symptom onset). The second blood specimen should be collected >72 hours after symptom onset.
Indeterminate	• It is unclear if nucleic acids from the virus of interest were present in the specimen. • This does not exclude infection and can arise for several reasons (e.g. only a single viral target was detected, inhibitory substances were detected, among others). • Confirmatory testing will be performed at the NML.	• Additional testing is recommended if the first specimen was indeterminate for a VHF-causing virus and was collected <72 hours after symptom onset AND if a VHF is still suspected on reassessment of the patient (e.g. no clinical improvement >72 hours after symptom onset). • The second blood specimen should be collected >72 hours after symptom onset.
Detected	• Nucleic acids from the virus of interest were detected in the specimen.Individual has an acute/recent infection with the virus tested. • If both BioFire and laboratory-developed tests were performed, either test resulted as “detected” is suggestive of infection. • Confirmatory testing will be performed at the NML.	• Patient has an acute infection with the VHF-causing virus • Other testing at PHO that is not essential for clinical management will be cancelled in consultation with the ordering physician.

[a]These results apply to all RT-PCR tests listed in Table 1.
[b]PHO results require confirmation by the NML.
[c]Laboratory confirmation requires: (i) detection of VHF-causing virus nucleic acids by RT-PCR at PHO and (ii) confirmatory testing performed by the NML.

Figure 1: Decision Tree for Repeat VHF RT-PCR Testing >72 Hours After Symptom Onset



For additional information on confirmation of VHFs, see Ontario Ministry of Health Infectious Disease Protocol Appendix 1: Viral Hemorrhagic Fevers

References

1. Ontario Agency for Health Protection and Promotion (Public Health Ontario). Viral hemorrhagic fever (VHF) symptom and exposure risk assessment: for clinician use [Internet]. Toronto, ON: King's Printer for Ontario; 2025 [cited 2025 Nov 7]. Available from: https://www.publichealthontario.ca/-/media/Documents/V/25/vhf-risk-assessment-checklist.pdf
2. Ontario Agency for Health Protection and Promotion (Public Health Ontario). Viral hemorrhagic fevers [Internet]. Toronto, ON: King's Printer for Ontario; 2025 [cited 2025 Nov 7]. Available from: https://www.publichealthontario.ca/en/diseases-and-conditions/infectious-diseases/vector-borne-zoonotic-diseases/ebola
3. Ontario Agency for Health Protection and Promotion (Public Health Ontario). Test information index [Internet]. Toronto, ON: King's Printer for Ontario; 2025 [cited 2025 Nov 7]. Available from: https://www.publichealthontario.ca/en/laboratory-services/test-information-index
4. Ontario. Ministry of Health. Public health management of viral hemorrhagic fevers – interim guidance [Internet]. Version 1. Toronto, ON: King's Printer for Ontario; 2022 [cited 2025 Nov 7]. Available from: https://www.ontario.ca/files/2024-05/moh-interim-viral-hemorrhagic-fevers-phu-guidance-en-2024-05-30.pdf
5. Biedenkopf N, Bukreyev A, Chandran K, Di Paola N, Formenty PBH, Griffiths A, et al. Renaming of genera Ebolavirus and Marburgvirus to Orthoebolavirus and Orthomarburgvirus, respectively, and introduction of binomial species names within family Filoviridae. Arch. Virol. 2023:168(8);220. Available from: https://doi.org/10.1007/s00705-023-05834-2
6. Public Health Agency of Canada. Ebola disease: for health professionals and humanitarian aid workers [Internet]. Ottawa, ON: Government of Canada; 2025 [cited 2025 Nov 7]. Available from: https://www.canada.ca/en/public-health/services/diseases/ebola/health-professionals-ebola.html
7. Centers for Disease Prevention and Control (CDC). Clinical screening and diagnosis for VHFs [Internet]. Atlanta, GA: CDC; 2024 [cited 2025 Nov 7]. Available from: https://www.cdc.gov/viral-hemorrhagic-fevers/php/laboratories/guidance-on-performing-routine-diagnostic-testing-for-patients-with-suspected-vhfs-or-other.html
8. Grolla A. Real-time and end-point PCR diagnostics for Ebola virus. In: Ebolaviruses. New York, NY: Springer New York; 2017. p. 341-52.
9. Mazzola LT, Cirino K. Diagnostics for Lassa fever virus: a genetically diverse pathogen found in low-resource settings. BMJ Glob Health. 2019;4(Suppl 2):e001116. Available from: https://doi.org/10.1136/bmjgh-2018-001116
10. Nikisins S, Rieger T, Patel P, Müller R, Günther S, Niedrig M. International external quality assessment study for molecular detection of Lassa virus. PLoS Negl Trop. 2015;9(5):e0003793. Available from: https://doi.org/10.1371/journal.pntd.0003793
11. Ontario. Ministry of Health. Ministry of Health emergency management plans and strategies [Internet]. Toronto, ON: King's Printer for Ontario; 2025 [cited 2025 Nov 7]. Available from: https://www.ontario.ca/page/ministry-health-emergency-management-plans-and-strategies
12. Pang Z, Li A, Li J, Qu J, He C, Zhang S, et al. Comprehensive multiplex one-step real-time TaqMan qRT-PCR assays for detection and quantification of hemorrhagic fever viruses. PLoS One. 2014;9(4):e95635. Available from: https://doi.org/10.1371/journal.pone.0095635

13. Ontario Agency for Health Protection and Promotion (Public Health Ontario). Viral hemorrhagic fever – including Ebola disease [Internet]. Toronto, ON: King's Printer for Ontario; 2022 [cited 2025 Nov 7]. Available from: https://www.publichealthontario.ca/en/Laboratory-Services/Test-Information-Index/VHF-Diagnostic-Serology
14. Ontario Agency for Health Protection and Promotion (Public Health Ontario). General test requisition [Internet]. Toronto, ON: King's Printer for Ontario; [cited 2025 Nov 7]. Available from: https://www.publichealthontario.ca/-/media/documents/lab/general-test-requisition.pdf?la=en
15. Public Health Agency of Canada. Biosafety guidelines for laboratories handling specimens from patients under investigation for Ebola virus disease [Internet]. Ottawa, ON: Government of Canada; 2023 [cited 2025 Nov 7]. Available from: https://www.canada.ca/en/public-health/services/infectious-diseases/viral-haemorrhagic-fevers/interim-biosafety-guidelines-laboratories-handling-specimens-patients-under-investigation-ebola-disease.html
16. Public Health Agency of Canada. Emergency Response Assistance Plans (ERAPs) [Internet]. Ottawa, ON: Government of Canada; 2024 [cited 2025 Nov 7]. Available from: https://tc.canada.ca/en/dangerous-goods/emergency-response-assistance-plans-eraps
17. Relich RF. High-consequence viral pathogens. In: Leber AL, Burnham CAD, editors. Clinical microbiology procedures handbook. 5th ed. Washington, DC: American Society for Microbiology; 2023.
18. Transport Canada. Shipping infectious substances [Internet]. Ottawa, ON: Government of Canada; 2024 [cited 2025 Nov 7]. Available from: https://tc.canada.ca/en/dangerous-goods/safety-awareness-materials-faq/industry/shipping-infectious-substances
19. Transport Canada. Appendix 3: guide to category A and category B assignment. In: Transportation of Dangerous Goods Regulations. Ottawa, ON: Government of Canada; 2025. Available from: https://laws-lois.justice.gc.ca/eng/regulations/SOR-2001-286/page-13.html#h-1228733
20. Government of Canada. Transport flowchart for risk group 4 pathogen(s) [Internet]. Ottawa, ON: Government of Canada; [2025] [cited 2025 Nov 7]. Available from: https://cnphi.canada.ca/gts/risk-4
21. World Health Organization (WHO). Diagnostic testing for Ebola and Marburg virus diseases: interim guidance [Internet]. Geneva: WHO; 2024 [cited 2025 Nov 7]. Available from: https://iris.who.int/server/api/core/bitstreams/e209d826-5f3d-4ca8-b278-69254569e7ac/content

Citation

Ontario Agency for Health Protection and Promotion (Public Health Ontario). Diagnostic testing for viruses that cause hemorrhagic fevers. Toronto, ON: King's Printer for Ontario; 2026.

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. This document may be reproduced without permission for non-commercial purposes only and provided that appropriate credit is given to PHO. No changes and/or modifications may be made to this document without express written permission from PHO.

Public Health Ontario

Public Health Ontario is an agency of the Government of Ontario dedicated to protecting and promoting the health of all Ontarians and reducing inequities in health. Public Health Ontario links public health practitioners, front-line health workers and researchers to the best scientific intelligence and knowledge from around the world.

For more information about PHO, visit publichealthontario.ca.

© King's Printer for Ontario, 2026

Ontario