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LABORATORY GUIDANCE

Diagnostic Testing for Viruses That Cause Hemorrhagic Fevers

Published: November 2025

Introduction

This document provides an overview of the process to test for high-risk viruses that can 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 high-risk viral pathogens. Refer to Public Health Ontario's VHF Symptom and Exposure Risk Assessment form and VHF Webpage for the relevant information.[1-2]
- Testing for other VHF-causing viruses not mentioned in this document, including vector-borne viruses (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 high-risk viruses that cause VHFs, including ED, may be indicated in individuals with compatible clinical signs, symptoms and exposures within a 21-day period prior to symptom onset. Consult Public Health Ontario's VHF Symptom and Exposure Risk Assessment form and Ministry of Health's Public Health Management of Viral Hemorrhagic Fevers – Interim Guidance for information relevant to clinical case review to determine if a patient meets criteria for a VHF.[1,4]

Key Considerations When Testing for Viruses Causing VHFs:

- The decision to proceed with VHF testing is a clinical decision made by the primary care team in consultation with provincial partners, including a PHO Microbiologist. The PHO Microbiologist will provide testing information relevant to the request, including any known limitations. It is the responsibility of the clinical team to ensure that the request for VHF testing is appropriate and in keeping with a suspected VHF.
- 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 travel and exposure history when determining the differential diagnosis. This information is required to support the request for VHF testing at PHO.
- 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.
- Due to biosafety concerns, both PHO's laboratory and the local/regional microbiology laboratory site that provides routine service to your institution should always be notified of any suspect case of VHF, especially if other specimens have already been collected that are in transit to the laboratory(ies) and if any testing has already been performed.
- VHF test requests require planning, notification and communication amongst all relevant stakeholders, including PHO, to ensure the safety of all parties involved.

Methods to Detect High Risk VHF-Causing Viruses at PHO

Testing by **real-time polymerase chain reaction (RT-PCR)** assays is preferred. These assays can detect viral nucleic acids in clinical specimens collected from individuals suspected of a VHF.[5-7]
PHO offers laboratory-developed RT-PCR tests for 7 high-risk VHF-causing viruses (Table 1).
Confirmatory testing is performed at the National Microbiology Laboratory (NML).

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

Orthoebolaviruses	Orthomarburgviruses	Other
Bundibugyo virus	Marburg virus	Crimean-Congo Hemorrhagic Fever virus
Ebola virus	Not applicable	Lassa virus
Sudan virus	Not applicable	Rift Valley Fever virus

- [a] Testing for other high-risk viruses not listed in Table 1 may be available upon request.
- [b] Submit the General Test Requisition along with specimens for VHF testing and list the virus(es) by their common name (as indicated in Table 1) in the Test(s) Requested field (e.g. Sudan virus PCR).

The RT-PCR protocols implemented at PHO were developed and validated at the NML and verified for clinical testing at PHO.[8,10,12] These are not multiplex PCR assays. Each RT-PCR protocol is performed as an independent test and detects one virus listed in Table 1. Most of the RT-PCR protocols detect two viral gene targets for one of the respective viruses listed in Table 1. The Sudan virus RT-PCR assay has only a single viral target. In accordance with federal guidelines, PHO laboratory results require secondary confirmation by the NML. The reporting plan will be communicated to clients at the time of submission.

PHO's laboratory will perform RT-PCR testing as soon as possible after specimens are received at PHO. The laboratory will also facilitate the safe and timely transfer of specimens from PHO to the NML for confirmatory testing. The turnaround time for all testing (both screening and confirmatory) will be communicated at the time of sample submission.

Where possible based on genome availability, PHO's laboratory will review RT-PCR primer sequences against publicly available databases to determine the likelihood that a currently circulating viral variant will be detected. Any limitations of the RT-PCR tests will be communicated in advance.

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 to PHO's laboratory due to biosafety risks.

Other Infectious Agents

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

Requesting VHF Testing from PHO's Laboratory

If the primary or other health sector organization feels that a patient's symptoms are compatible with a suspected VHF, the Ministry of Health should be immediately notified via the Health Care Provider Hotline (1-866-212-2272). They will facilitate coordination of the provincial (including local health unit) and federal health systems to respond to the situation in accordance with the High-Risk Pathogen Notification Pathway.

Contact PHO to alert them of the request if suspecting any of the viruses listed in Table 1:

- PHO Customer Service Centre: (416-235-6556 or 1-877-604-4567) during regular laboratory hours

OR

- PHO Duty Officer (416-605-3113) after hours

A PHO Microbiologist or other designated staff member will follow-up with the requestor to confirm the test(s) requested and provide relevant information regarding testing. Once a PHO Microbiologist agrees to testing, PHO's laboratory will follow-up with the submitter regarding next steps, including specimen collection and transport. See sections below for additional information on these items.

Requirements for VHF Testing at PHO

Activities Prior to Specimen Collection

Prior to Collecting Specimens for VHF Testing:

- Discuss the case with your local laboratory management team to ensure that any specimens for testing are collected and transported in accordance with the Transportation of Dangerous Goods Act/Regulations and any non-VHF testing not performed at PHO or the NML is performed safely in accordance with a local risk assessment.[19] This includes transporting specimens between laboratory sites.
- Inform PHO's laboratory of the intent to collect specimens for VHF testing. This includes a discussion with a PHO Microbiologist and other provincial testing partners.
- Collect only specimens that are essential for diagnosis and monitoring. Specimens should be collected by staff experienced in the required techniques following recommended safety procedures including proper use of personal protective equipment (PPE).
- All laboratory staff (both hospital/local laboratory and PHO) should be alerted to the specimen information. Once received, the specimens should remain in the custody of designated persons from the time of specimen receipt until testing is complete.

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 VHF testing after consulting with provincial partners, including a PHO Microbiologist, the specimens listed in Table 2 should be collected and sent to PHO in accordance with requirements outlined by Transport Canada for Emergency Response Assistance Plans (ERAP).
- If both VHF testing **AND** malaria testing is required based on the differential diagnosis, refer to both the Viruses causing VHF and Malaria requirements in Table 2.
- If only VHF testing is required, refer only to the Viruses causing VHF specimen requirements in Table 2.

Table 2: Specimen Requirements for High-Risk VHF-causing Viruses and Malaria Testing

Pathogens of Interest	Tests Performed at PHO	Specimen Information[a,b,c]
Viruses causing VHF:[13] • Bundibugyo virus • Crimean-Congo Hemorrhagic Fever virus • Ebola virus • Lassa virus • Marburg virus • Rift Valley Fever virus • Sudan virus	RT-PCR	**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 **If the patient is an INFANT or if collection is difficult:** • At least 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] Other specimen types may be acceptable but have uncertain clinical performance. Safely collect the appropriate number of specimens for testing and label with a minimum of two patient identifiers. PHO's laboratory will not aliquot samples from patients under investigation for high-risk viruses.

Each specimen submitted to PHO for VHF testing should be accompanied by its own separate PHO laboratory General Test Requisition, requesting only the specific VHF agent(s) for which testing has been arranged and clearly stating the patient's suspected diagnosis and risk factors. Specimens with non-VHF tests requested on the same requisition will be cancelled.[14]

If additional tests (e.g. malaria) are requested of PHO laboratory, separate specimens must be submitted (Table 2), each with its own PHO laboratory General Test Requisition, clearly stating the patient's suspected diagnosis and risk factors. Requests for non-essential microbiology tests sent to PHO's laboratory are subject to cancellation.[14]

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 following the initial onset of symptoms .[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 cases confirmed by RT-PCR may be indicated and should follow institutional policies. The WHO and others recommend that two negative RT-PCR tests from blood samples collected 48 hours apart should be considered.[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 Recommendations
Whole Blood	Less than 72 hours	Not Detected	• This does not rule out VHF infection • A second collection for repeat RT-PCR testing (and malaria smear) is recommended if a VHF is still suspected when the patient is re-assessed at least 72 hours after symptom onset (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 is needed 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 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 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 and confirm if an ERAP is needed. Additional information on biosafety and specimen handling 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. 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.

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.

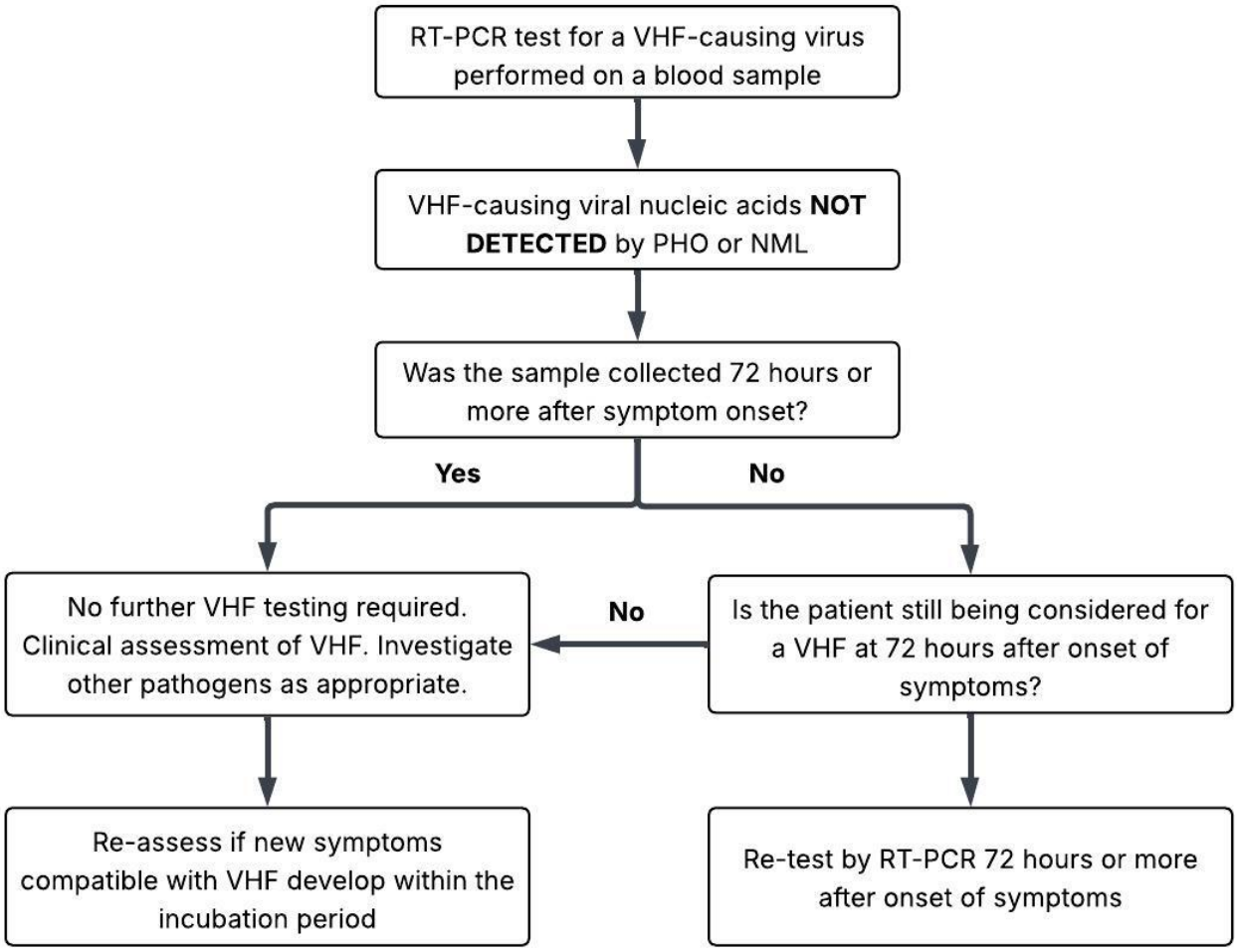
All VHF test results should be interpreted with caution and in the appropriate clinical and epidemiological context (Table 4). Repeat testing may be warranted in some scenarios based on the timing of specimen collection. Refer to Table 4 and Figure 1 for more information.

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 viral nucleic acids were not detected from a specimen collected >72 hours after symptom onset and the result was confirmed by the NML. Other testing appropriate for patient care can 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 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.
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. • 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 primary care team.

- [a]These results apply to all RT-PCR tests listed in Table 1.
- [b]The PHO results are preliminary and require confirmation by the NML.
- [c]A laboratory-confirmed VHF infection requires: (i) detection of the genetic targets of a particular virus in an RT-PCR by PHO and/or (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 laboratory confirmation of VHFs, see Ontario Ministry of Health Infectious Disease Protocol Appendix 1: Viral Hemorrhagic Fevers

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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; 2025.

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