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LABORATORY GUIDANCE

Diagnostic Testing for Viruses That Cause Hemorrhagic Fevers

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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 Public Health Ontario (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 process and methods used
- Specimen requirements
- Specimen processing
- Result interpretation and additional testing

As of June 2026, PHO has enhanced laboratory testing for viruses that cause hemorrhagic fevers with the implementation of an additional multiplex molecular assay, the Global Fever RUO Panel. This document has been updated to provide information on this new assay and revised testing details.

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 VHF 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, and yellow fever virus). Information on diagnostic testing for these viral pathogens can be found on Public Health Ontario's Laboratory Test Information Index.[3]
- Conducting local laboratory biosafety risk assessments for tests not performed at PHO that are essential to patient care. Refer to Public Health Ontario's Clinical Laboratory Biosafety Measures in Viral Hemorrhagic Fever Investigations resource for guidance and considerations prior to undertaking these assessments.

PHO Testing in the Investigation of Suspected VHF

Testing for VHF-causing viruses is available through PHO's laboratory by special request. In cases where a VHF is suspected, consult Public Health Ontario's VHF Symptom and Exposure Risk Assessment form to guide the clinical assessment, and to identify subsequent actions to take once it has been completed. Refer to the Ontario Ministry of Health's Notification Pathway for Special Pathogens for a description of the provincial coordination process for special pathogens, including VHF-causing viruses, which may be activated if there is a strong suspicion of a VHF following completion of the risk assessment and an institutional decision to proceed with testing.[1,4] Where warranted, they may facilitate coordination of the provincial and federal health system partners, including PHO, to respond to the situation.

Key Considerations When Requesting Testing for Viruses That Cause VHFs:

- Testing for VHF-causing viruses requires a careful balance between establishing the pre-test probability, biosafety measures and the efficient use of laboratory and health system resources.
- Clinical teams should ensure that prior to activating the provincial response pathway, any request for VHF testing is justified based on all relevant information that is suggestive of VHF.
    - The likelihood of a VHF should be assessed in the context of the patient's clinical presentation, including onset date, together with relevant travel and exposure history. Travel details should include specific locations visited, particularly areas with known or ongoing transmission of VHF-causing viruses. This information is required to support the testing request and inform the differential diagnosis.
    - Other, more common and potentially life-threatening infectious diseases - such as malaria, typhoid fever, and bacteremia - should also be considered, as appropriate in individuals presenting with a compatible illness.

Methods to Detect VHF-Causing Viruses at PHO

Real-time polymerase chain reaction (RT-PCR) is the primary testing method used to identify VHF-causing viruses. These assays detect viral nucleic acids in clinical specimens obtained from patients under investigation for a VHF.[5-7] PHO performs two independent RT-PCR testing approaches to detect VHF viruses, a commercial assay (FilmArray Global Fever RUO Panel) and laboratory-developed tests (LDT). When VHF testing is requested, the VHF viruses in question should be ordered individually based on the clinical risk assessment.

The **FilmArray Global Fever RUO Panel** is a multiplex RT-PCR assay that enables the rapid, simultaneous detection of multiple pathogens in a single test (Table 1). PHO has recently evaluated this assay in collaboration with the National Microbiology Laboratory (NML) and other provincial laboratories in the Canadian Laboratory Response Network (CLRN). The assay has been implemented at the PHO Toronto and PHO London laboratories to enhance laboratory support for suspected VHF cases. It is only available in the context of VHF investigations and cannot be ordered as a standalone test for the detection of any non-VHF pathogens on the panel.

The **LDT RT-PCR assays** in use at PHO were initially developed and validated by the NML to detect the viruses listed in Table 2. Unlike the FilmArray Global Fever RUO Panel, these tests are not multi-pathogen multiplexed assays and instead detect nucleic acids only of a specific virus.[8,10,12] PHO's laboratory reviews LDT RT-PCR primer sequences to verify whether VHF viral variants associated with active outbreaks are detectable by the LDT RT-PCR assays (where possible, based on genome availability in public sequence repositories).

Serologic testing using enzyme-linked immunosorbent assays (ELISA) is also available through the NML for the pathogens listed in Table 2. However, serology is of limited value in the diagnosis of acute VHF infection. Antibodies typically develop several days after symptom onset, reducing the usefulness of serology testing to inform timely clinical decision-making. Serologic testing may be useful to diagnose individuals presenting later in the disease course (e.g. convalescent phase) with compatible symptoms.[23]

Table 1: Pathogen Targets in Global Fever Panel Multiplex PCR Assay[a,b]

Viruses	Bacteria	Other
Chikungunya virus	*Bacillus anthracis*	*Leishmania* spp.
Crimean-Congo Hemorrhagic Fever virus	*Francisella tularensis*	*Plasmodium* spp.
Dengue virus	*Leptospira* spp.	*Plasmodium falciparum*
Orthoebolaviruses[c]	*Salmonella* Paratyphi A	*Plasmodium vivax/Plasmodium ovale*[d]
Lassa virus	*Salmonella* Typhi	-
Marburg virus	*Yersinia pestis*	-
West Nile virus	-	-
Yellow Fever virus	-	-
Zika virus	-	-

[a] This assay has been evaluated in the context of VHF investigations. Extensive testing was not performed for all non-VHF targets in the multicentre evaluation, except for *Plasmodium* spp. (malaria). For this reason, results are reported for investigational use. Non-VHF targets with 'Detected' results will be confirmed by further testing using PHO's standard methods, where necessary.
[b] Tests for RG4 VHF-causing viruses not listed in Table 2 may be available upon request.
[c] Nucleic acids of orthoebolaviruses are detectable, but the assay cannot determine the specific virus. Additional LDT testing at PHO and the NML will provide this information.
[d] The assay is unable to discriminate between *P. vivax* and *P. ovale*.

Table 2: Laboratory Developed RT-PCR Tests for VHF-Causing Viruses Available at PHO[a,b]

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

[a] Testing for RG4 VHF-causing viruses not listed in Table 2 may be available upon request.
[b] The LDT RT-PCR assays used will be guided by the clinical risk assessment and must be specified at submission
[c] Identification of clinically relevant Orthoebolaviruses is possible with the LDT RT-PCRs
[d] Testing for Rift Valley Fever virus is only performed at PHO by LDT. It is not included in the FilmArray Global Fever RUO Panel

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 and Other Differential Agents of Febrile Illness in Travelers:

Malaria testing is available at PHO's laboratory and will be performed in VHF investigations. The FilmArray Global Fever RUO Panel will detect *Plasmodium* species (malaria), including *P. falciparum* and *P. vivax/P. ovale*. Other routine malaria testing methods will also be performed if the FilmArray Global Fever RUO Panel result is positive for any of the malaria targets or if this assay is unavailable.

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

The FilmArray Global Fever RUO Panel can also detect the other potential co-pathogens listed in Table 1. 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. Additional testing will be performed at PHO for any non-VHF target that produces a positive result.

Other Infectious Agents:

Other non-essential microbiological testing, except for malaria, will not be performed at PHO until the RG4 virus under investigation has been ruled out. This includes tests that require culture and any other non-viral tests that are non-essential for acute patient management.

For tests that are not offered by PHO, such as routine blood cultures for bacterial analysis, it is recommended that only limited non-VHF microbiology testing that is necessary for patient management be performed. This is only possible if a local laboratory biosafety risk assessment has been conducted and your local laboratory has decided on an approach that allows testing to be performed safely.

It is recommended to consult the hospital/community laboratory that provides microbiology services to your institution to determine if they have the capability to perform this testing in this setting in accordance with institutional policies. Information to support local laboratory biosafety risk assessments for tests essential for patient care that are not performed at PHO is available in Public Health Ontario's Clinical Laboratory Biosafety Measures in Viral Hemorrhagic Fever Investigations resource provides.[22]

Requirements for VHF Testing at PHO

Activities Prior to Specimen Collection

Prior to collecting specimens for VHF testing:

- The case should be discussed with your local laboratory management team both for awareness and to ensure that 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.[15,19,22]
- If the decision to proceed with VHF testing was confirmed during a Ministry of Health-organized provincial health system partners coordination call, ensure that the local/regional microbiology laboratory providing routine services to your institution is notified, if not already aware, of any specimens from the patient that were collected and tested or collected and are already in transit for non-VHF microbiology testing before an RG4 VHF was included in the differential diagnosis.
- Similarly, confirm with your local microbiology laboratory if any specimens have already been recently referred to or tested by PHO, as residual specimen may be acceptable for use in VHF testing. In this case, additional collections may not be required.

Specimens to Collect and Submit for VHF Testing

Only those specimens listed in Table 3 should be submitted to PHO for VHF testing. 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 Guidance for Viral Hemorrhagic Fever Specimen Collection and Handling in Acute Care Settings and your institutional guidance and policies for additional information around the safe collection of specimens in the clinical environment.

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.

Table 3: Specimen Requirements for RG4 VHF Viruses and Malaria Testing

Pathogens of Interest	Tests Performed at PHO	Specimen Information[a,b,c,d]
RG4 viruses causing VHF:[13] • Bundibugyo virus • Crimean-Congo Hemorrhagic Fever virus • Ebola (Zaire) virus • Lassa virus • Marburg virus • Sudan virus	**RT-PCR** (FilmArray Global Fever RUO Panel and LDTs)	**Requirements:** • 2 x EDTA-blood tubes for VHF testing • One tube will be tested by RT-PCR at PHO • One tube will be forwarded to the NML **For ADULTs:** • 2 to 4 mL of blood is required per tube **For PEDIATRIC patients or if collection is difficult:** • 0.5 to 1 mL of blood is required per tube
Malaria	**RT-PCR** (FilmArray Global Fever RUO Panel) Other routine malaria tests if positive by FilmArray Global Fever RUO Panel	**Requirements:** • 1 x EDTA-blood tube for malaria testing • 2 to 4 mL blood is required in the tube

[a] Tubes should **not** be opened or pretreated prior to shipping to PHO.
[b] **DO NOT** submit pre-made thick and thin smear slides on patients under investigation for a VHF.[22]
[c] **DO NOT** use glass specimen collection devices/containers, unless there is no other alternative. [22]
[d] Whole blood is the preferred specimen type.[21,23] The clinical performance of other specimen types is uncertain.

Key Considerations for Specimen Collection:

- The timing of specimen collection is important for interpretation of the Global Fever RUO Panel and VHF LDT RT-PCR results.[17,23] Viral nucleic acids in blood may not reach levels that are detectable by laboratory tests until 72 hours after symptom onset, therefore specimens collected very early in illness may yield negative results.[7,8,21]
- Each specimen submitted to PHO listed in Table 3 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 for specific VHF causing viruses that have been discussed with health systems partners should be listed, and the patient's suspected diagnosis and risk factors clearly stated. Specimens with non-essential microbiology tests requested will be cancelled.[14] Refer to PHO's Test Information Index for additional information.

Shipping Specimens to PHO for VHF Testing

Specialized training/certification, packaging, transportation and documentation is required when transporting between hospital sites and VHF testing laboratories, including PHO.[15,18-19,24]

Key Considerations When Shipping Specimens for VHF Testing:

- PHO's laboratory is available to support your site with shipping packages to PHO (and the NML, if appropriate). **Refer to Public Health Ontario's** Ground Shipment of Clinical Specimens to PHO for Viral Hemorrhagic Fever Investigations **for step-by-step guidance for safely shipping and transporting clinical specimens to PHO**.[24] This information can be used to supplement your institutional processes, if necessary.
- Specimens suspected or confirmed to contain RG4 VHF virus listed in this document are subject to Part 7 of the federal Transport Canada Transportation of Dangerous Goods (TDG) Regulations. These requirements include special packaging, handling and shipping provisions, including the need for an Emergency Response Assistance Plan (ERAP).[16,18,19 24] An ERAP is a Transport Canada-approved plan that outlines the procedures, specialized expertise, equipment and response actions required when an actual or anticipated release of high-risk dangerous goods occurs during transport.[18] The ERAP is activated when the first shipment or package is picked up and remains active until the last shipment or package reaches its destination. When multiple packages are transported together, only a single ERAP activation is required. Refer to the NML's Transport Flowchart for Risk Group 4 Pathogen(s) for a general overview of the ERAP process and Transport Canada for additional requirements, beyond the scope of this document.[18, 20]
- It is the submitting site's responsibility to ensure that staff handling specimens are TDG certified and a courier service that is certified to transport ERAP agents has been pre-arranged.
- 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.
- Only ship the specimens outlined in Table 3 to PHO. Do not include specimens for other non-essential tests or specimens from other patients. Category A packaging and materials sent to PHO will not be returned to the submitter.

VHF Testing and Result Interpretation

VHF Testing Process at PHO

Specimens are processed, handled and tested as soon as possible once they are received at PHO. PHO's VHF testing algorithm includes the use of **both** the FilmArray Global Fever RUO Panel and the VHF LDT RT-PCR assays.

Testing will be initiated using the FilmArray Global Fever RUO Panel. The VHF LDT RT-PCR assay for the suspected VHF-causing virus(es) indicated on the PHO General Test Requisition will be performed in all cases, regardless of the FilmArray Global Fever RUO Panel result. If the FilmArray Global Fever RUO Panel detects nucleic acids from a non-VHF pathogen, additional testing will be conducted at PHO as appropriate (e.g. the Dengue, Chikungunya, Zika virus multiplex RT-PCR will also be performed).

In parallel, PHO's laboratory will support the timely transfer of a specimen to the NML for additional VHF testing in accordance with federal guidelines.[23] This will occur without waiting for the PHO test results to be released. Turnaround times for VHF testing vary depending on specimen receipt time, transportation logistics, and requirements for additional testing.

Interpretation of VHF RT-PCR Results

All VHF laboratory test results require interpretation alongside the available clinical and epidemiological information (Table 4). The reporting plan will be communicated to the submitter at the time of specimen receipt at PHO. Results will be released as soon as possible to the ordering clinician indicated on the General Test Requisition and the appropriate public health unit.

A qualitative result will be provided for all targets on the Global Fever RUO Panel and VHF LDT RT-PCRs in accordance with Table 4. The two-method VHF testing algorithm at PHO provides additional support for a comprehensive assessment of suspect cases. Any testing limitations will be communicated by the PHO Microbiologist during a health system partner coordination call arranged by the Ontario Ministry of Health as part of the Notification Pathway for Special Pathogens.

Additional RT-PCR testing is performed at the NML to enable testing and reporting in accordance with WHO International Health Regulations.[23]

Interpretation of Non-VHF Pathogen RT-PCR Results

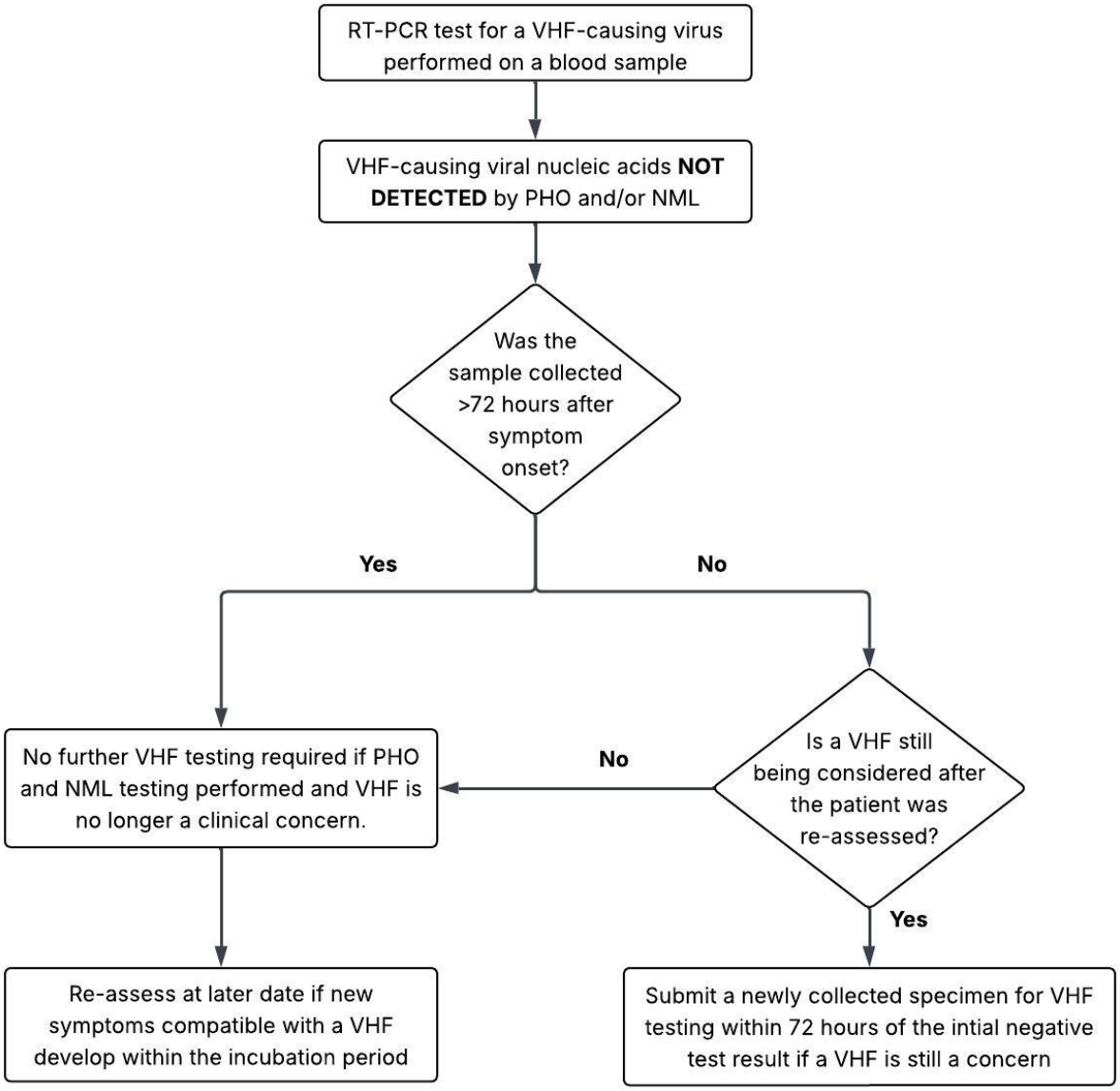
The Global Fever RUO Panel includes testing for non-VHF pathogens, including malaria. As the multicentre evaluation of this panel was limited in scope, all non-VHF pathogen targets with a positive ('Detected') result will be confirmed, where necessary, using PHO's standard laboratory methods. No additional testing will be performed for non-VHF pathogen targets with a negative ('Not Detected') result. Refer to PHO's Test Information Index for pathogen-specific testing information.

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

PHO VHF RT-PCR Result[a,b]	Interpretation of PHO Result	Follow-Up
Not Detected	• Nucleic acids from the virus(es) tested were not detected in the specimen. • PHO results are preliminary. Additional testing will be performed at the NML. • A negative RT-PCR result alone does not exclude infection and should be interpreted in conjunction with clinical and epidemiologic information to determine subsequent actions on a case-by-case basis.	• No further VHF testing is recommended if the blood specimen was collected >72 hours after symptom onset and a clinical decision has been made to conclude the VHF investigation.[23] Other pathogens should be investigated if an infectious cause is still suspected. • A repeat VHF test on a new blood collection may be considered if the initial specimen produced a negative result and was collected <72 hours after symptom onset AND the clinical suspicion of VHF remains high following re-assessment of the patient (e.g. no clinical improvement or the onset of new, compatible symptoms[c]).[7,17,23] This specimen can be collected within 72 hours of the initial negative test result (Figure 1)
Indeterminate	• It is unclear if nucleic acids from the virus of interest were present in the specimen. • PHO results are preliminary. Additional testing will be performed at the NML. • This result does not exclude infection and can arise for several reasons (e.g. only one VHF LDT RT-PCR target was detected, the PCR reaction was inhibited by unknown substances in the blood).	• A repeat VHF test on a new collection may be considered if an indeterminate result was obtained for both the PHO and NML result from a patient with a high index of suspicion for a VHF. • If the indeterminate result is only from PHO tests, repeat testing should await the NML result, where possible.
Detected	• Nucleic acids from the virus of interest were detected in the specimen. • PHO results are preliminary. Additional testing will be performed at the NML. • Results are suggestive of an acute infection with the VHF-causing virus that was detected by Global Fever RUO Panel and/or PHO VHF LDT RT-PCR.	• Other testing at PHO that is not essential for clinical management may be cancelled in consultation with the ordering clinician.

[a]Results apply to all targets in Table 1 and Table 2. Indeterminate results only apply to PHO's VHF LDT RT-PCR.
[b] The Global Fever RUO Panel provides a generic Orthoebolavirus result and does not differentiate these viruses.
[C] Reconsider 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.

Figure 1: Decision Tree for Repeat VHF RT-PCR Testing



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

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