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LABORATORY GUIDANCE

Clinical Laboratory Biosafety Measures in Viral Hemorrhagic Fever Investigations

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Purpose

This document provides biosafety guidance for clinical laboratories handling specimens from patients under investigation for viral hemorrhagic fever (VHF), including disease caused by risk group 4 viruses such as orthoebolaviruses (e.g., Bundibugyo virus). Its purpose is to support timely clinical testing needed for patient assessment and care, while minimizing risk to laboratory personnel and limiting unnecessary disruptions to laboratory operations. This guidance is intended to inform local risk assessments and operational decisions in diagnostic clinical laboratory settings.

Background

Viral hemorrhagic fevers (VHFs) are a group of severe, often life-threatening infectious diseases caused by viruses within several families, including *Filoviridae* (e.g., Bundibugyo virus, Ebola virus, Sudan virus, and Marburg virus), *Arenaviridae* (e.g., Lassa virus), and *Nairoviridae* (e.g., Crimean-Congo Hemorrhagic Fever virus). This document focuses primarily on the VHFs of greatest biosafety concern in the clinical laboratory setting, particularly those associated with risk group 4 (RG4) pathogens, as per the Human Pathogen and Toxins Act.[1]

These viruses may be transmitted to humans from infected animals (e.g. fruit bats) or from direct contact with blood and other body fluids from infected individuals. Early illness is often non-specific and may include fever, fatigue, weakness, headache, nausea, vomiting, and diarrhea. The incubation period ranges from 2-21 days, and persons are not infectious until symptom onset.[2,3] Progression of the disease can result in worsening gastrointestinal symptoms, organ dysfunction and hemorrhagic manifestations. There are currently no approved vaccines or specific antiviral treatments for many VHF-causing viruses, including Bundibugyo virus.[4] Because many patients under investigation ultimately have alternative diagnoses, clinical evaluation and essential laboratory testing should proceed promptly while appropriate precautions are maintained.

Scope

This guidance applies to hospital and clinical laboratories that may receive, collect, transport, process, test, store, or dispose of specimens from patients under investigation for VHF. It addresses routine diagnostic testing performed within CL2 (or higher) laboratories required for patient management, specimen handling and transport, containment and work practices, decontamination, spill response, waste management, and referral of specimens for specific VHF testing. This is suggested guidance that is intended to support local risk assessments when tailoring biosafety practices to your facility. As a result, practices may vary between sites. It does not replace institutional procedures, public health requirements, or applicable biosafety and Transportation of Dangerous Goods (TDG) Regulation.[5]

- For public health case and contact management, refer to the Ontario Ministry of Health's Public Health Management of Viral Hemorrhagic Fevers – Interim Guidance[6]
- For specific VHF testing information, refer to PHO's Laboratory Guidance on Diagnostic Testing for Viruses That Cause Hemorrhagic Fevers[7]
- For IPAC guidance, refer to PHO's Infection Prevention and Control Management of Viral Hemorrhagic Fever in Acute Care[8]
- Testing of 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[9]

Clinical Course

Patients under investigation for VHF typically present with a compatible clinical syndrome together with an epidemiologic risk factor, such as relevant travel or exposure history. Early disease manifestations are often non-specific, and many patients are ultimately found to have other illnesses such as malaria, influenza, typhoid fever, or other bacterial or viral infections. For this reason, rapid clinical assessment (e.g., following the VHF Clinical Risk Assessment Tool) and access to laboratory and diagnostic testing are essential and should not be delayed while VHF is being considered.[10] Appropriate infection control precautions should be followed when caring for patients with suspected or confirmed VHF infection.[8]

Where VHF is part of the differential diagnosis, clinicians should activate the notification pathway for special pathogens to discuss whether specific VHF testing is indicated and safe practices for patient management.[6] Laboratories should be prepared to support urgent patient care needs during this assessment period.

Transmission in Healthcare and Laboratory Settings

VHF viruses are transmitted through contact with infected blood, body fluids, tissues, or contaminated materials, and in some instances through exposure to infected animal reservoirs. In healthcare and laboratory settings, the principal concern is exposure through splashes, sprays, sharps injuries, direct contact with mucous membranes or broken skin, and aerosol-generating procedures involving infectious material. The risk associated with clinical diagnostic laboratory work is considered low when appropriate engineering controls, work practices, and personal protective equipment (PPE) are used.[21]

Recommended Laboratory Testing

Patients with compatible symptoms and relevant epidemiological risk factors should be managed using appropriate infection control precautions. Once the decision to proceed with testing is confirmed through the provincial special pathogens notification pathway, VHF testing should be facilitated as soon as possible after symptom onset, alongside other investigations guided by the clinical presentation and differential diagnosis.[8,6,9]

Repeat testing for VHF-causing viruses may be considered when initial nucleic acid amplification test results are negative, especially if the first sample was collected less than 72 hours after the onset of symptoms, clinical suspicion remains high, and the patient's condition is deteriorating with no alternative diagnosis.[2,9]

Testing should be limited to what is necessary for patient management and should be selected based on the clinical presentation, travel history, institutional capability (including available equipment and reagents), and ability to adhere to appropriate infection prevention and control and biosafety measures. Testing workflows should be designed to reduce manipulation, aerosol generation, and instrument contamination. In addition to VHF testing, the following laboratory investigations may be considered for patients under investigation, where specimen collection and testing can be safely performed based on local risk assessments to support appropriate clinical management and maintain continuity of care while awaiting VHF test results.

Microbiology

- Blood cultures for bacterial pathogens
- Malaria testing

Chemistry

- Electrolytes
- Creatinine
- Blood glucose
- Liver function tests
- Blood gases

Hematology

- Complete blood count (CBC), including differential and platelet count
- Coagulation testing, including prothrombin time/international normalized ratio (PT/INR)

Biosafety Risk Assessment and Mitigation

A biosafety risk assessment is a process used to identify: 1) the hazards associated with a known or potentially infectious agent and the activities being conducted with them; 2) the likelihood of a person's exposure to that agent or material; and 3) the consequences of such an exposure to personnel or equipment (e.g., a laboratory acquired infection or the need to quarantine an instrument for extended periods).

An assessment of the risk associated with all processes, procedures, and activities in the laboratory must be performed to determine the potential for exposure to the specimen through generation of aerosols, sprays, splashes, or spills. Based on the assessment, a plan to mitigate the identified risks should be implemented using engineering controls, administrative controls (including work practices), and use of appropriate PPE (see below). The WHO's framework is a recommended resource for conducting local risk assessments.[15]

Specimen Collection Instructions

- Specimens should be collected only when clinically indicated and by personnel trained in both specimen collection and the required infection prevention and control and biosafety precautions.[13]
- The number of specimens collected should be minimized to those required for immediate patient care and VHF tests.[2,9]
- Tubes and requisitions should be clearly pre-labelled before collection whenever possible, and specimens from patients under investigation for VHF should be clearly identified in accordance with institutional policy.
- Current recommendation for specific VHF testing includes collecting 2 EDTA tubes of blood for PCR and a third EDTA tube for malaria testing where indicated.[2,9]
- After collection, the exterior of primary specimen containers should be inspected for contamination and always decontaminated before transport. Specimens should be placed in a durable, leak-proof secondary container (see below) and transported by hand; pneumatic tube systems should not be used.
- Collection staff should ensure that the receiving laboratory (on site or off site) has been notified in advance when specimens from a patient under investigation for VHF are being sent.

General Biosafety Guidance for Specimen Handling and Processing

- If capability at a local facility exists then specimens for essential diagnostic testing do not need to be delayed, referred out, or canceled while awaiting VHF testing results if handled according to appropriate biosafety measures and local risk assessments.
- At minimum, specimens from patients under investigation for VHF should be handled in a facility that meets the requirements for containment level 2 (CL2) as per the Canadian Biosafety Standard, together with a completed local risk assessment and additional precautions appropriate to the hazards identified.[11]
    - Core principles include strict adherence to CL2 laboratory practices, minimizing specimen manipulation, restricting access to testing areas to authorized personnel, avoiding sharps wherever possible, using validated decontamination procedures, and ensuring that staff are both trained and competent in the tasks they perform including PPE donning and doffing.
- Perform all procedures with the potential to generate aerosols, sprays, or splashes inside a certified biological safety cabinet (BSC) class II or other appropriate primary containment device.
- Use sealed centrifuge cups or sealed rotors and open them only inside a BSC class II.
- Limit testing to designated trained and competent personnel, designated equipment, and designated work areas.
- Where possible, limit traffic, clutter and personnel in the designated work areas
- Store specimens securely and ensure they are accessible only to authorized personnel.
- Incorporate engineering controls, administrative controls, safe work practices, and appropriate PPE as part of the overall risk mitigation strategy.

Considerations for Specific Enhanced VHF Precautions in the Diagnostic Laboratory

Because VHF specimens may contain high-consequence pathogens, laboratories should include enhanced precautions in addition to baseline CL2 practices.[3,15-22] Staff should use a combination of engineering controls, work practices and PPE to protect their mouth, nose, eyes and bare skin from contact with patient specimens. This includes the proper use of containment devices, specifically, BSC class II and PPE within clearly identified designated areas in CL2 spaces at a minimum.

Activities should be planned to minimize aerosol generation, unnecessary movement of specimens, use of sharps, and presence of unnecessary personnel in the area. A buddy system, clear entry and exit procedures, and advance training in donning and doffing may further reduce risk.

Enhanced PPE for Specimen Testing Include (as per PHAC)[15]:

a. two pairs of gloves (e.g., latex, nitrile, or other similar material);

b. an additional impermeable layer over the lab coat (e.g., fluid-resistant gown with extended cuffs);

c. eye and respiratory protection, such as a combination of an approved particulate respirator (e.g., N95 or N100) and eye protection (e.g., goggles, face shield, or shroud), or a powered air purifying respirator (PAPR).

PPE should be removed in the presence of a trained observer, in a manner that minimizes contamination of the skin and hair, and avoids any contact between soiled items (e.g., gloves, gowns, respirators) and any area of the face. When removing the outer layer of double gloved hands, use alcohol-based hand rub to sanitize the inner gloves, prior to putting on a new set or prior to removing the remaining set. Hands should be washed thoroughly immediately after the removal of PPE.

Blood Cultures

a. Prepare blood culture bottle handling in BSC class II and label appropriately for easy identification when placed in an automated blood culture incubator system.[15] Assess risks associated with transferring blood culture bottles from BSC class II to incubator and consider additional measures such as a disposable or wipeable transport box and/or trolley.

b. Sub-culturing of blood cultures has the potential to generate aerosols and should be done only when essential to patient care. If proceeding, this should be done in a certified BSC class II with the use of additional PPE as identified above. When Gram stains are performed, slides should be fixed prior to staining, with a method that inactivates VHF viruses (e.g., in 100% methanol for at least 15 minutes to inactivate VHF viruses).

Malaria

As per the Committee to Advise on Tropical Medicine and Travel (CATMAT), preliminary test results for suspect malaria cases should be available within two hours of blood draw, otherwise empiric malaria treatment may have to be considered.[12]

a. **Thin blood smear microscopy:** All manipulations should be performed in a certified BSC class II with the use of appropriate PPE as identified above. After air drying (without fan) in a certified BSC class II, thin blood smears should be fixed with 100% methanol for at least 15 minutes.[18] Thick blood smears should not be done due to the inability for full methanol inactivation.[18] All reagents should be decontaminated prior to disposal (e.g., using bleach) or otherwise disposed of as category A liquid waste. If a site does not perform expert malaria microscopic identification, slides should not be forwarded to other reference sites for confirmation due to the risk of sharp injury with slide transportation; instead, sites should forward the whole blood tube to the reference site.

b. **Antigen testing:** alternative method if molecular testing is not available locally. Similar as above, may be performed in its entirety within a certified BSC class II with appropriate PPE or following inactivation outside of the BSC. Both positive and negative results should ideally undergo thin smear microscopy due to the variable accuracy of antigen tests.

c. **Rapid molecular testing (e.g., LAMP or PCR):** preferred method to use where available due to its superior sensitivity. May be performed in its entirety within a certified BSC class II with appropriate PPE as identified above, or the blood may be inactivated (e.g., with guanidine salt and ethanol inactivation, or alternatively heat followed by Triton X-100 inactivation) prior to handling outside of the BSC. Positive results should ideally undergo species identification and parasitemia by thin smear microscopy (see below).

Biochemistry and Hematology

a. It is preferred to use fully enclosed devices with limited sample preprocessing requirements (e.g., devices intended for point-of-care or near-patient use) to perform essential non-microbiology testing within a CL2 laboratory. See appendix A for a list of device examples. The instrument should be placed in a BSC class II and operated by laboratory personnel trained on appropriate PPE donning and doffing and instrument use. Ensure that the instrument and other supplies do not impede airflow in the BSC. All devices should be cleaned and decontaminated according to the manufacturer's instructions for use. After use, devices should be decontaminated and should not leave the designated testing area between use. For use with laboratory-confirmed VHF positive specimens, devices that cannot be decontaminated internally may require removal from use and handled based on local risk assessment in collaboration with the manufacturer.

b. If an institution chooses to use automation lines, careful evaluation should include vendors as part of the risk assessment team to determine the aerosol generating risks and the ability to appropriately clean and decontaminate the instrument. Routine use of automation lines that have the potential to generate aerosols (e.g., centrifugation, decapping) should be avoided unless the risk of aerosolization and contamination is low following local risk assessment and the sample is fully traceable and processed separately from other routine samples. Alongside other controls and enhanced practices, recommend inactivating samples if used in automation lines outside of other biosafety containment measures (e.g., BSC).

Molecular Testing

Molecular testing such as polymerase chain reaction (PCR), if available on-site, may be considered a safe option based on local risk assessment. Extraction procedures (e.g., guanidine thiocyanate-based supplemented with ethanol) are sufficient to inactivate VHF viruses. Nucleic acid extractions should be undertaken in a certified BSC class II.

Transportation Within and Outside the Facility

Within the Facility

Primary containers should be handled using appropriate PPE as per IPAC guidance.[13] Following Category A transportation regulations, specimens should be tripled packaged, in sample biohazard bag(s) and placing in a durable, leak-proof secondary container with absorbent material for transport. Consider placing the secondary container in a transport bag for ease of carrying the specimens. Transport routes should be pre-planned to avoid high-traffic areas where feasible. Pneumatic tube systems should not be used for specimens from patients under investigation for VHF.

Outside the Facility

For transportation outside the facility, Transport Canada classifies VHF viruses as Category A.

Thus, clinical specimens from patients at risk for VHF infection should be transported as Category A infectious substances (UN 2814, infectious substance, affecting humans) in accordance with applicable regulatory and institutional requirements. Federal shipping and transportation regulations, including the requirement to activate an Emergency Response Activation Plan (ERAP), must be followed when handling or transporting specimens for VHF testing.[14] All specimens must be shipped in compliance with the Transportation of Dangerous Goods Act.[5] Refer to Laboratory Guidance Diagnostic Testing for Viruses That Cause Hemorrhagic Fevers for information on the transport and shipment of specimens to PHO for VHF testing.[7]

Laboratories should maintain a log of all individuals who have handled, decontaminated and transported these types of clinical specimens, including waste associated with the specimens.

If transportation delays are expected, samples should be refrigerated or frozen at -70°C, except for sample tubes intended for malaria testing. Contact PHO's Laboratory Customer Service Centre (416-235-6556 during business hours or Duty Officer 416-605-3113 after hours) for assistance in packing and transporting samples outside of the facility.

Shipping Samples to the National Microbiology Laboratory:

- Packaging, shipping and transport of specimens must comply with the requirements of the Transportation of Dangerous Goods Regulations, Transport Canada and the Dangerous Goods Regulations, International Air Transport Association.[15-16]
- For shipments, patient/primary sample specimens should be shipped as (UN2814, Category 6.2), and Emergency Response Assistance Plan (ERAP) must be initiated.[14]
- Liaise with the provincial public health laboratory of your jurisdiction to coordinate with the National Microbiology Laboratory (NML) Operations Center Director (OCD) at 1-866-262-8433. The OCD is staffed 24/7.
- The NML OCD will work with the requesting provincial jurisdiction to initiate the ERAP. If you require assistance with the shipping process, sample requirements, sample shipping conditions, the NML OCD will connect you with the appropriate subject matter experts.
- Concurrent with a request for laboratory services for Ebola or other viral hemorrhagic fevers, provinces and territories are requested to notify and provide a clinical history of the patient's illness to the Public Health Agency of Canada Health Portfolio Operations Centre (HPOC) at 1-800-545-7661. Clarification or further information may be requested from the patient's clinician to optimize the delivery of the requested laboratory service(s).

Cleaning, Disinfection, and Decontamination

Work surfaces, equipment, specimen containers, and other potentially contaminated items should be cleaned and disinfected using hospital-approved disinfectants. Follow manufacturer's instructions for use and local institutional policies and procedures for cleaning and disinfection guidance.[3, 15-19]

Contaminated and potentially contaminated clothing and PPE are to be decontaminated using an effective method. Refer to your local institutional polices and procedures to safely manage Category A solid waste.

Decontamination of Equipment (e.g., POCT Devices used in BSC Class II)

For decontamination of laboratory instruments and equipment, use of a Health Canada-registered hospital-approved disinfectant with label claims for non-enveloped viruses for cleaning and decontaminating surfaces or objects is recommended.

The laboratory should consult in advance with the manufacturer to ensure the most appropriate selection of such disinfectants and their use on the equipment. Some disinfectants can be detrimental (i.e., corrosive) to the instrument's surface.[3, 15-19]

The manufacturer instructions should be consulted to see what the manufacturer recommends when taking the equipment out of commission or preparing for maintenance or repairs.

If an instrument is contaminated during use and there is no procedure for decontamination of the internal compartments without compromising the instrument operability, then the instrument may need to be removed from service and assessed as there may be no methods for ensuring that remaining viral particles within the instrument are no longer viable.

Spills Management

For spills, the area should be secured immediately and access restricted. Personnel involved in cleanup should wear task-appropriate PPE and follow the local spill response procedure. Visible material should be absorbed and removed using tools that minimize direct contact, after which the affected area should be cleaned and disinfected using an appropriate disinfectant and contact time. Materials used in cleanup should be treated as infectious waste.

Limit the number of personnel involved in the clean-up and develop protocols for safely remediating spills containing broken glass.

Before any spill clean-up is initiated, ensure staff are trained and wear recommended PPE as above to protect against direct skin and mucous membrane exposure of cleaning chemicals, contamination, and splashes.

All materials used for cleanup must be treated as Category A infectious materials and disposed of in a biohazard waste container according to local policies and procedures.

Management and Disposal of Biomedical Waste for Risk Group 4 Pathogens (e.g., Ebola Virus) in Diagnostic Laboratories

Orthoebolaviruses are classified as a Category A infectious substance thus all potentially contaminated liquid and solid materials generated during the handling and testing of specimens must be contained to prevent leakage or puncture and appropriately decontaminated before disposal, reuse or removal from the laboratory.[11,15]

Manage VHF-associated biomedical waste using a precautionary approach: segregate at the point of generation, package as close as possible to where it is generated, limit handling to trained personnel, keep sealed containers closed, and treat waste before final disposal.

Validated treatment processes such as autoclaving or incineration may be used, where appropriate, to render associated waste non-infectious before final disposal. Laboratory-generated biomedical waste effectively treated using a validated process may then be managed under facility procedures for treated, non-infectious waste.

Residual specimens should be managed according to existing institutional procedures for RG4 pathogens. Consult the local or provincial public health laboratory for direction, including whether referral or destruction is appropriate.[5, 11, 16]

Each facility should perform local risk assessments and verify compliance with internal procedures and applicable requirements.

Occupational Health

Potential exposures to these specimens must be reported immediately according to your institution's policies and procedures. Licensed facilities are to report exposures to the PHAC without delay. Facilities that are not regulated by the PHAC may do so on a voluntary basis. Exposure reports may be submitted online or by email to pathogens.pathogenes@phac-aspc.gc.ca.

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Appendix A

Table 1: Examples of Fully Enclosed Hematology and Biochemistry Point of Care Devices for Consideration to be Used in CL-2 or Higher Laboratory Setting

Tests	Point of Care Device									
	Abbott iSTAT	Siemens EPOC	Roche Inform II	Nova Statstrip	Piccolo Xpress	Roche Coaguchek Pro II	Werfen Hemochron	Siemens Poch-100i	Sysmex XQ-320	Siemens Clinitek Status Plus
Blood Gases	x	x								
Electrolytes (sodium, potassium, chloride, total CO2)	x	x			x					
Creatinine	x	x			x					
Glucose	x	x	x	x	x					
Liver function (AST, ALT, GGT, ALP, bilirubin)					x					
PT-INR						x	x			
CBC								x	x	
Urinalysis										x

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