4. Laboratory Diagnosis of Viral Haemorrhagic Fever Viruses

Contents

Early infection with VHF is usually characterised by a short non-specific viral prodrome with an extensive differential diagnosis. The presumptive diagnosis of VHF is based on epidemiological and clinical assessment with laboratory testing used to confirm or exclude the diagnosis.

Molecular and serological protocols are used to diagnose infection with arenaviruses, filoviruses and bunyaviruses in containment level 3 (CL3) facilities. In Ireland, a molecular diagnostic service is offered by the National Virus Reference Laboratory (NVRL) located at University College Dublin (UCD), Dublin for detection of the following Biosafety 4 viruses - Filoviruses (Ebola group viruses and Marburg virus), Crimean Congo Haemorrhagic Fever Virus, Lassa Virus and also orthopox viruses. Confirmation, when required is performed at the Rare and Imported Pathogens Laboratory (RIPL) located at Public Health England (PHE) Porton, Salisbury, U.K. using molecular and/or serological assays

This chapter provides a description of the diagnostic tests recommended for ***At Risk with NO high risk exposure*** and **high risk exposure** patients and details the specimen collection requirements for VHF-specific tests. Packaging and transport instructions for referral to the laboratory are also included. Clinical specimens derived from patients with VHF are classified as **Category A** infectious substances. It is ESSENTIAL that the clinical laboratory that processes these tests are informed of the suspicion of VHF, so that specimens can be segregated and processed separately using dedicated equipment where appropriate. Each laboratory should have a risk assessed contingency plan for dealing safely with potential VHF specimens which includes out-of-hours operational procedures and contact details for key personnel.

The information in the chapter is based on guidance from the Emerging Viral Diseases Laboratory Network EVD-LabNet, from the Advisory Committee on Dangerous Pathogens (ACDP) and from Public Health England (PHE).

4.1 Risk assessment

Key Points

- For specimens from all patients in whom VHF is being considered, an appropriate local risk assessment **must** be undertaken, including an evaluation of the risks associated with each analytical technique and the application of relevant control measures

- Upon presentation of a possible case of VHF in the ***At Risk with NO high-risk exposure*** category, MALARIA tests should be performed immediately. If the malaria test result is NEGATIVE, consider VHF testing.

Practical Note:

- Laboratory investigation for VHF is only performed following prior consultation with the NVRL. Consultation is available out of hours by calling 01-7164050. During normal business hours, the NVRL telephone number for queries is 01-7164401.
- Request forms for investigation of VHFs can downloaded from the NVRL website and must be completed in full before testing can proceed.

Patients presenting to hospital with fever and a history of recent travel to an area where there is an on-going VHF outbreak or the VHF is endemic undergo a risk assessment (usually in the Emergency Department), to determine their level of VHF risk. For more information, please see VHF Guidance Chapter 2: *Clinical Assessment, Risk Categorisation and Management in Acute and Primary Care settings* on the HPSC website here.

On the basis of this risk assessment patients are divided into two groups:

1. **At risk *with NO high risk exposure*** (low risk exposure category i.e. they have not had any direct exposure to blood or body fluids from a known VHF case, or other high risk exposure). Such patients are very unlikely to have VHF.
2. ***High risk exposure*** (i.e. they have had direct exposure to blood or body fluids from a known VHF case, or other high risk exposure). Such patients are considered to have a significant risk of having VHF, though the majority will not have VHF.

This risk assessment must be carried out by clinical staff, and laboratory staff must be made aware of the patient's risk classification, before samples are referred to the laboratory for urgent investigations required for immediate patient management. For all patient specimens with a risk of VHF, specific risk assessments must be developed alongside local codes of practice, which should be agreed between clinical and laboratory staff before the laboratory can accept samples.

This information can be used to ensure that the risks are effectively controlled and relevant facilities are in place and are managed properly. The risk assessment should include evaluation of the risks associated with each analytical technique, in particular those which may generate aerosols and the application of appropriate control measures. Although any cause of fever must be considered in a febrile patient suspected of VHF infection, the major infectious microbial causes include malaria, shigellosis, typhus or typhoid fever.

4.2 Referral and delivery of specimens to diagnostic laboratories

Key Points

- Consultation with the laboratory is **ESSENTIAL** prior to sampling. Only specimens essential for immediate patient management and diagnosis should be obtained for investigation.
- Each laboratory should have a risk assessed contingency plan for dealing with potential VHF specimens which includes out-of-hours operational procedures and contact details for key personnel.
- Within the hospital, specimens should be transported and tested according to local risk assessed arrangements for high-risk samples.
- Personnel involved in referral of samples should receive appropriate training.

It is ESSENTIAL that hospitals have procedures in place to ensure laboratory staff are informed prior to samples from a patient considered to be at risk of VHF ('At Risk with NO high risk exposure or 'high risk exposure') or with confirmed VHF, being delivered to the laboratory for investigation. The level of VHF risk should be clearly stated on the request form and sample label. Only samples essential for immediate patient management or diagnosis should collected

Samples should be hand-delivered to the laboratory in a sealed container. Pneumatic tube systems should not be used to deliver samples from patients considered to be at risk of VHF or with confirmed VHF to the laboratory.

4.3 Packaging and transport of biological specimens

Viruses causing haemorrhagic fevers are classified as **Category A** infectious pathogens.

Within the hospital, specimens should be transported according to local risk assessed arrangements for high-risk samples. Precautions should include:

- primary containers must be leak-proof and a waterproof, leak-proof seal must be used;
- secondary containers should be placed in a good quality box, which is securely taped closed and clearly labelled "Pathological Specimen - Open only in Laboratory";
- specimens should be transported by hand by a responsible person using the above packaging. Pneumatic tube systems **must not be used** for transportation of specimens within hospitals or laboratories;
- specimens should not be processed in the routine specimen reception area.

For transport from the pathology laboratory to the reference laboratory, specimens should be packaged in UN2814 certified packaging and transported according to UN602 guidance. Regulations regarding packaging and transport of Category A infectious substances are governed the ADR (European Agreement concerning the International Carriage of Dangerous Goods by Road) and International Air Transport Association (IATA), both of which use the United Nations Model Regulations system.

Personnel involved in packaging and sending samples are responsible for adhering to current regulations and interpreting applicable regulations for their facility. Appropriate certified training is recommended.

Each local laboratory should have appropriate packaging on site. Category A transfers should be individually requested through an approved courier. The courier must be licensed to carry dangerous goods and have appropriate training. The service should be available 24/7 and must involve tracked door-to-door delivery, which must be signed for on collection and receipt.

Specimens must be transported in triple packaging system according to the following instructions:

- primary containers must be leak-proof and a waterproof, leak-proof seal must be used;
- the secondary packaging must also be leak-proof and contain sufficient absorbent material to absorb the entire contents of the primary container. If multiple primary containers are packaged together they must be individually wrapped to prevent contact;

- the outer shipping packaging should be UN2814 certified (Packaging Instruction 602);
- specimen data forms, letters etc. should be taped to the secondary container. Request forms are available at the NVRL website.

Confirmatory testing on all samples with a positive result generated at the NVRL ARE carried out at the RIPL. Transport of samples to the RIPL will be coordinated by the NVRL according to UN602 guidance.

4.4 Laboratory testing

(a) *At Risk with NO high risk exposure* (low risk exposure category)

- Emergency testing for malaria can be carried out using a WHO-approved rapid diagnostic test at the bedside following risk assessment but should be followed up as soon as possible with blood film analysis by experienced laboratory staff in a microbiological safety cabinet (MSC) at containment level (CL)2.
- VHF testing is ONLY performed following prior consultation with the NVRL and upon receipt of a completed VHF investigation request form.
- . If the VHF screen is negative then the possibility of the patient having a VHF infection should be maintained until an alternative diagnosis is confirmed.

Only specimens essential for patient management or diagnosis should be obtained for investigation. **Appropriate PPE** must be worn during specimen collection. Standard Precautions should be applied when sampling from a patient categorised as **At Risk with NO high risk exposure**. If the patient is symptomatic then Standard plus Contact plus Droplet Precautions should be applied.

The preferred specimen for diagnosis of VHF is blood and urine. Ideally, 5ml blood is required to complete the VHF analysis although 1ml volume is sufficient to perform essential tests where sufficient sample volume is difficult to obtain (e.g. infants and young children). The NVRL investigates specimens using molecular diagnostics. However, supplementary confirmatory testing such as serological investigations is provided by RIPL. Diagnosis by detection of viral genome is suitable for patients in the early stage of illness, while serological diagnosis by the detection of specific IgM and IgG antibodies is suitable for patients in a relatively late stage of illness. Acute-phase specimens should be collected within 7 days of illness onset. Convalescent-phase specimens should be collected 7-20 days later, and at least 14 days after illness onset. It is essential that recent travel, contact history, clinical manifestations and date of onset are provided to the NVRL prior to sample collection to determine which investigation is appropriate

Table 1**. Laboratory tests required for **At Risk with NO high risk exposure

Recommended Laboratory Tests

• A thin blood smear (EDTA blood specimen) to look for malaria parasites on at least two occasions
  ○ Thick films should not be prepared
• Two sets of blood cultures, using routine blood culture bottles, from separate vein puncture sites taken at least 30 minutes apart
  ○ 20 to 30ml per set (5-10ml volumes are appropriate for children)
• White blood cell and differential count and either haemoglobin or haematocrit
• Renal profile (Urea & electrolytes)
• Urine culture, if urinalysis results suggest an infection
• Glucose measurements
• Liver functions tests
• Prothrombin time (PT) and Activated Partial Thromboplastin Time (APTT)

If the VHF screen is negative then the possibility of the patient having a VHF infection should be maintained until an alternative diagnosis is confirmed.

(b) For high risk exposure category patients

Key points

- Liaison with the local microbiologist/virologist or infectious disease physician is essential prior to requesting VHF-specific diagnostic tests.
- VHF testing is carried out only following prior consultation with the NVRL and upon receipt of a completed VHF investigation request form.
- Initial processing of samples collected for molecular investigations must be carried out at CL-3 level. If specimens are inactivated, molecular investigations can be performed at CL2.
- A malaria screen and all other analyses listed in Table 1 should also be conducted urgently using CL-2 procedures with additional precautions as outlined below, following **a detailed risk assessment** of the procedures and equipment to ensure safe investigation. Any procedures that are likely to generate aerosols should be carried out in a certified biological safety cabinet.
- Laboratory staff dealing with specimens from patients with suspected VHF must take, as a minimum, the same personal protective precautions as patient-care staff and must have experience dealing with samples which may have a high viral load
- If results are negative then the possibility of the patient having a VHF infection should be maintained until an alternative diagnosis is confirmed.

Following clinical assessment if the patient is classified as **high risk exposure category** then VHF-specific diagnostic tests should be carried out urgently These tests should be carried out concurrently with a malaria screen and all other analyses listed in **Table 2** in facilities that have risk assessed these procedures. Liaison with the local microbiologist/virologist or infectious disease physician is ESSENTIAL

Laboratory staff dealing with specimens from patients categorised as **high risk exposure category** must take, as a minimum, the same personal protective precautions as patient-care staff (see Chapter 3).

If the VHF screen is negative then the possibility of the patient having a VHF infection should be maintained until an alternative diagnosis is confirmed. Infection control precautions should also be maintained.

***Table 2**. Laboratory tests required for **high risk exposure category** patients*

Recommended Laboratory Tests

• A thin blood smear (EDTA blood specimen) to look for malaria parasites on at least two occasions
  ○ Thick films should not be prepared
• Two sets of blood cultures, using routine blood culture bottles, from separate vein puncture sites taken at least 30 minutes apart
  ○ 20 to 30ml per set (5-10ml volumes are appropriate for children)
• White blood cell and differential count and either haemoglobin or haematocrit
• Renal profile (Urea & electrolytes)
• Urine culture, if urinalysis results suggest an infection
• Glucose measurements
• Liver functions tests
• Prothrombin time (PT) and Activated Partial Thromboplastin Time (APTT)

4.5 Recommended laboratory biosafety precautions

(a) Processing samples from patients considered to be At Risk with NO high risk exposure (low risk exposure category)

Patients who have a fever (or history of fever) plus a history of travel to a country currently affected by a VHF outbreak (within the previous 21 days), but have no high risk exposures **are very unlikely to have VHF**. Such patients are far more likely to have malaria, typhoid, shigellosis, or other infections, rather than VHF.

The greatest risk to such patients is failure to diagnose a treatable infection, or complication of infection, due to delays in processing routine laboratory diagnostic samples.

Samples from such patients can be safely processed using routine laboratory biosafety precautions (CL-2) in facilities that have-risk assessed these procedures. However, some additional precautions are recommended, as outlined below:

a. Specimens can be processed at CL 2 in routine autoanalysers.
b. Waste from autoanalysers is not considered to pose a significant risk because of the small sample size and dilution step and therefore requires no special waste disposal precautions.
c. Procedures must be in place for the effective management of spillages.

d. A sealed centrifuge bucket or rotor should be used for centrifugation procedures that are being undertaken manually (i.e. not within an autoanalyser).
e. Preparation of blood film slides for malaria testing (thin blood film only, no thick blood film) and any procedures that are likely to generate aerosols should be carried out in a certified biological safety cabinet. Laboratory staff carrying out such procedures should wear the following personal protective equipment (PPE):
    i. Fluid repellent full sleeved gown
    ii. Gloves
    iii. Closely fitted respirator-type mask (FFP2 or FFP3)
    iv. Eye protection (goggles or face shield)
f. Fixed/dried blood film slides can be safely examined at CL 2 outside of a biological safety cabinet.
g. Blood cultures can be processed in automated instruments. However, sub-culturing of blood culture bottles should be carried out in a certified biological safety cabinet, using the PPE detailed above.

Laboratory staff dealing with specimens from patients categorised as **At Risk with NO high risk exposure (low risk exposure category)** must take, as a minimum, the same personal protective precautions as patient-care staff. The level of protection required, including PPE, depends on the patient's symptoms and the procedures being performed. Details are provided in Chapter 3.

(b) Processing samples from patients considered to be high risk exposure category or from patients with confirmed VHF

Patients who have a fever, plus a history of travel to a country or region currently affected by an VHF outbreak **and** have **high risk exposures** are classified as being at **high risk** of VHF (although most patients in this category will not have VHF). If VHF is considered to be a likely diagnosis or if **VHF is confirmed**, the patient may be transferred to the National Isolation Unit (NIU) at the Mater Misericordiae University Hospital, Dublin. In such cases, laboratory testing will be carried out at the NIU.

Specimens from patients categorised as having **high risk exposure** to VHF, or with **confirmed VHF**, can still be processed safely in a CL 2 laboratory that have risk assessed these procedures, in particular aerosol generating procedures. Regardless of whether or not a patient is to be transferred to the NIU, specimens from patients classified as having a **high risk exposure** to VHF, or with **confirmed VHF,** should be processed promptly in a CL 2 laboratory if required for immediate patient care, with the following additional precautions:

a) Senior laboratory staff should be available to manage the coordination of testing and liaise where appropriate with other laboratories.
b) Specimen cuvettes from routine autoanalysers should be safely disposed of as category A waste.
c) A local risk assessment should be carried out for test protocols not undertaken in routine autoanalysers that are likely to result in the production of splashes or aerosols. Where appropriate, these tests should be undertaken in a certified biological safety cabinet. Staff carrying out such tests should wear the following PPE:

i. Fluid repellent full sleeved gown
ii. Gloves
iii. Closely fitted respirator-type mask (FFP2 or FFP3)
iv. Eye protection (goggles or face shield)

d) For manual centrifugation procedures, a sealed centrifuge bucket or rotor must be used.
e) Patient samples that are not for immediate disposal should be packed in rigid containers, which should be surface decontaminated and retained within the laboratory while awaiting safe disposal.
f) Disinfection and decontamination procedures, validated as effective against blood-borne viruses, must be in place.
g) Autoanalyser disinfection procedure should be carried out following sample processing and before scheduled maintenance.
h) Work surfaces should be treated with 1,000 ppm available chlorine promptly after completing work on the sample.
i) If VHF is confirmed, retained specimens must be processed as Category A waste. Where possible, specimens should be inactivated by autoclave on site, and subsequently can be processed as Category B waste. Where this is not possible, they should be securely transported as Category A waste for destruction by incineration.
j) If VHF is confirmed, blood film slides should be disposed of in a dedicated sharps bin, which must be processed as category A waste (as above).

4.6 Reporting of results

- Preliminary VHF specific test results will be issued to the requesting clinician. Reactive samples are referred to RIPL for confirmation.
- Test results on **high risk exposure** patients will also be reported to the Director of Public Health/MOH.
- The MOH will also inform the IHR National Focal point at HPSC of the result
- Printed reports are issued when all laboratory tests have been completed, typically within 3-6 days.

4.7 Disposal of residual blood samples

All laboratory waste generated during investigation of an **At Risk with NO high risk exposure** VHF case should be treated as Category B infectious waste. Waste in this category must be packaged in accordance with P621 or LP621 or IBC620 of ADR regulations.

All laboratory waste generated during investigation of a suspected **high risk exposure** VHF case should be treated as **Category A waste** and autoclaved and/or incinerated. Waste in this category must be packaged in accordance with P620 of ADR regulations. The transport, storage and arrangements for disposal of waste need to be carried out by named persons. All personnel involved in this process should be competent, specifically trained and wear appropriate PPE.

4.8 Risk of laboratory-acquired infection

For individuals with VHF, the highest concentration of virus is found in blood or secretions. However, the virus may be present in other body fluids (including urine, faeces, respiratory tract secretions, semen etc.). The principal risk for acquisition of infection, when working with samples that may contain VHF virus, is percutaneous inoculation injury. Exposure of mucous membranes to splashes of infectious material, and inhalation of infectious aerosols, are also considered potential modes of acquisition. However, there is no circumstantial or epidemiological evidence of any aerosol transmission risk from VHF infected patients.

When standard laboratory biosafety procedures are adhered to, the risk of laboratory-acquired infection with VHF is extremely low. Cases of laboratory-acquired infection with viruses causing VHF have been reported, mainly in virology laboratories or laboratories working with potentially infected animals.

VHF viruses are readily inactivated using disinfectants routinely used in diagnostic laboratories. Decontamination protocols that inactivate enveloped viruses (such as influenza or hepatitis C) will also inactivate VHF viruses.

Current ACDP and CDC guidance state that **processing samples from patients with suspected or confirmed VHF using routine biosafety precautions in diagnostic laboratories poses no greater risk than samples containing hepatitis B, hepatitis C, HIV and other blood-borne viruses.**

4.9 Contact information

Table 3. *Contact Information for National Virus Reference Laboratory (NVRL) and the Rare and Imported Pathogens Laboratory (RIPL), U.K.*

	Address	**24/7 Emergency Telephone number**	**Request Form**
National Virus Reference Laboratory	University College Dublin Belfield Dublin 4, Ireland	00 353 1 7164401 (Office hours) 00 35317164050 (24/7 emergency number)	https://nvrl.ucd.ie/sites/default/files/uploads/pdfs/NVRL_VHF_Investigation_Request_Form_LF_UM_001d_4.pdf
Rare and Imported Pathogens Laboratory	HPA Porton, Salisbury, Wiltshire, SP4 OJG, U.K.	00 44 1980 612100 (24/7 emergency number) 00 44 844 7788990 (24/7 medical advice only)	https://www.gov.uk/government/publications/rare-and-imported-pathogens-testing-form-to-submit-sample