

Technical Report Series
***Laboratory Procedures and Precautions for Samples
Collected from Patients with Viral Haemorrhagic
Fever***

Prepared by the Public Health Laboratory Network

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Part A Guidelines for laboratories not associated with a designated isolation hospital
(amended and republished October 2014).

Part B Guidelines for laboratories associated with a designated isolation hospital (amended
and republished October 2014).

Part A

Guidelines for laboratories that are not associated with a designated isolation hospital

These recommendations are designed to provide guidelines for laboratories at hospitals other than the designated state or territory VHF isolation hospital. It is anticipated that they will only be involved in the care of a suspected case until such time as they can be transferred to the designated hospital. The work carried out should be the minimum necessary for the management of the patient. These guidelines should be shared with hospital personnel responsible for policy writing for managing critical care patients.

It is the responsibility of the laboratory director to ensure that the state or territory laboratory servicing the designated hospital is aware that samples may be forwarded to them for testing. Alternatively laboratory directors from smaller state or territory jurisdictions should alert the designated reference laboratory in a larger jurisdiction whose services they use. This must be done as early as possible to allow them to prepare for these samples, and to obtain advice about specimen handling and processing.

Contact details can be obtained from the Chief Quarantine Officer for the state or territory, or can be found in the state or territory VHF Response or Contingency Plan.

Introduction

There are a large number of viruses that cause viral haemorrhagic fever (VHF), but only a few are known to pose a risk of transmission within healthcare settings. This group of VHF comprises Lassa fever (LF), Congo-Crimean haemorrhagic fever (CCHF), Ebola virus and Marburg virus. These viruses are found in Africa and, in the case of CCHF, in some adjacent areas. Only Marburg virus has been shown to cause an outbreak of human disease in a developed country. This occurred in 1967 when laboratory workers in Marburg, Germany became infected when handling kidneys from African green monkeys. A single case of a laboratory worker becoming infected with Ebola has also been described.

Transmissions from patients to healthcare workers within modern healthcare settings occurred during the Marburg virus outbreaks and possibly from a case of Ebola virus disease in South Africa, but they are rare. However because of this potential and because these diseases have high mortality rates, there are stringent recommendations for the care of suspected cases in Australia. These four VHFs are proclaimed quarantinable diseases under the Quarantine Act 1908. If VHF is suspected, the Chief Quarantine Officer for the state or territory must be notified immediately. Details of the procedure for the management of a VHF case can be found in each state or territory's VHF Response or Contingency Plan and the *Guidelines for the Management of Human Quarantine Disease in Australia*. Each State and Territory has designated hospitals for receiving these patients that are equipped to provide the necessary standard of care.

VHF may be suspected in a wide range of situations varying from asymptomatic contacts through all the phases of illness, or even a retrospective diagnosis after the patient has recovered or died.

Also, patients presenting with suspected VHF are uncommon in Australia and the initial clinical presentation may be non-specific (fever, pharyngitis, and myalgia, with or without haemorrhagic manifestations). As a result, recognition of VHF cases may be slow, and they may have been cared for in institutions other than a designated hospital, and diagnostic samples may have been sent to more than one laboratory before the diagnosis is suspected or confirmed. High standards of routine patient care and specimen handling should offer good protection, however assessment and surveillance of staff that have had contact with the patient or samples would be necessary. Fortunately patients with advanced and highly infectious diseases are more likely to be clinically recognised and transferred to a designated hospital. Also past Australian experience with suspected VHF cases show that most are caused by other infectious agents, in particular malaria, and there have been no cases of confirmed VHF in Australia.

Hospitals and laboratories designated for the clinical care and diagnosis of cases of VHF face a number of problems. As suspected cases are rare, patient care and laboratory facilities are generally used for other purposes, and these must be made available for VHF cases at short notice. Also, there are potentially a wide range of tests required for these patients, and as these cannot all be performed within a specified area, routine laboratories have to be used. With rare exceptions, laboratories do not have access to Physical Containment (PC) 4 facilities and, usually, do not have PC3 facilities. Therefore the precautions to be used must be based on enhancing protection within a PC2 environment.

Persons under investigation who do not meet the case definition of suspected case can be managed as per routine laboratory practices.

Scope of Testing and Collection of Specimens

1. A risk assessment of the likelihood of VHF should be undertaken prior to the collection of specimens in conjunction with infectious diseases and public health physicians.
2. On-site testing should be kept to the minimum necessary for the management of the patient.
3. Wherever possible specimens should be collected at predetermined 'routine' times to allow the laboratory to plan for specimen processing. The receiving laboratory should be notified in advance before specimens are dispatched.
4. Specimen tubes, receptacles, containers, canisters and a specimen request form should be labelled prior to the collection of specimens. Appropriate personal protective equipment must be worn. Glass specimen containers must not be used. Disposable sharp objects, such as scalpel blades or needles must be placed in a rigid sharps container immediately after use and must be sterilised in an autoclave or incinerated. During collection every effort must be taken to avoid external contamination of the specimen tube or container.
5. It may be appropriate for laboratories to supply clinicians with pre-packed specimen collection kits with the required blood tubes, containers, canisters with detailed instructions on specimen collection and packing as part of VHF preparedness. An example for packing specimens is described:-
 - i. Each blood tube or primary receptacle is wiped with 0.5% (5000 ppm) sodium hypochlorite solution and placed into a container (e.g. 50 mL BD Falcon™ Tube [polyethylene flat-top screw cap] for blood tubes), with absorbable material placed between the primary receptacle and the container.
 - ii. The container is then placed into a biohazard specimen bag, with the request form inserted into the outer pocket of the specimen bag or attached using plastic tape if a bag without an outer pocket is used. Under no circumstances should the request form be placed in the same container as the specimen, nor should it be attached with pins or staples.
 - iii. The specimen bag is then placed into a canister (e.g. Bio-Bottle [<http://www.biobottle.com.au>]) and the canister is wiped with 0.5% (5000 ppm) sodium hypochlorite solution before transport to the laboratory.
6. Specimens must be transported to the laboratory in clearly labelled ("Infectious Risk") appropriate containers. These will contain the specimen if the blood collection tube should leak. Specimens must not be sent by any automatic transport system (e.g. pneumatic air tube system). The laboratory will then package the specimen for referral to a reference laboratory where VHF testing is performed. Specimens should be transported in accordance with current regulatory requirements.
7. Ebola virus and Marburg virus are classified as Tier 1 Security Sensitive Biological Agents (SSBA), and specimens should be handled and transported to the testing laboratory in accordance with the regulatory requirements.

Laboratory Receipt and Processing

1. Where on-site processing is necessary, a separate room or area containing a Class 1 or 2 Biosafety Cabinet (BSC) with a HEPA filter on the air exhaust should be designated to receive the samples. Samples that have not been inactivated must only be handled in the BSC.

2. BSCs must be cleaned after spills and at the end of the working session. They should be wiped over with 1% glutaraldehyde or 0.5% (5000 ppm) sodium hypochlorite solution and left to dry with the extraction system running. The room will need to be vacated until the odour has dissipated.
3. In case of extensive contamination of the room it must be sealed and decontaminated with formaldehyde gas.
4. Personnel involved in handling laboratory specimens must be kept to a minimum. It is preferred that competent senior staff are designated to process these samples. Nonessential staff should vacate the area when samples are being processed. Pregnant, immunosuppressed or immunocompromised staff members are not permitted to work with specimens from patients with suspected VHF.
5. A written record of all personnel involved in laboratory testing must be kept with dates, times and analyses performed recorded. A logbook will be placed in the laboratory for use by all staff handling specimens. This will be the responsibility of the nominated senior scientist.
6. Ten millilitres (10 mL) of clotted blood must be collected from staff handling specimens from patients subsequently confirmed to have VHF which must be stored for baseline serology.
7. Laboratory staff handling non-inactivated specimens must wear full protective clothing consisting of double gloves, impervious long-sleeve gowns, shoe covers, N95/P2 masks and the full-face visor. Overalls must be worn under the impervious long-sleeve gowns, and these should be disposable if available. The full-face visor is unnecessary if working in a class 1, 2 or 3 Biological Safety Cabinet (BSC). After completing a session of work with the specimens, all disposable protective clothing should be sealed in a thick plastic bag, and disposed of by incineration. If incineration is not possible clothing, sealed in an appropriate plastic bag, should be autoclaved. The effectiveness of sterilisation should be documented by a Bowie-Dick test. The sterile garments may then be disposed of with routine hospital medical waste. If non-disposable overalls were used, and are not visibly soiled, then they should be soaked in 1% (10000 ppm) sodium hypochlorite for 10 minutes in the laboratory before being sent for laundering. If they are visibly soiled, then they should be incinerated. Face visors should be immersed in 0.5% (5000 ppm) sodium hypochlorite solution for 10 minutes, washed and dried for re-use if they are contaminated or at the end of each shift in which they have been used. Hands must be washed after leaving the room with Betadine (if iodine-allergic, chlorhexidine or 70% (w/v) alcohol) under running water.
8. Non-inactivated specimens can be processed in automated analysers that do not require removal of the top of the blood collection tube, provided there is proper disposal of waste fluids and the machine can be decontaminated after use. Otherwise, a suitably experienced individual in the designated receiving area (DRA) must perform tests using manual methods.

Automated machinery should be decontaminated with 0.5% (5000 ppm) sodium hypochlorite solution for several cycles and the external surfaces wiped over with 0.5% (5000 ppm) sodium hypochlorite solution. If the manufacturers recommend an alternative decontamination procedure, then it must be verified that it is adequate to inactivate the agents of the VHFs. If the process is known to be sufficient for the inactivation of Hepatitis C and/or Hepatitis B virus, then it will be adequate for the viruses causing VHFs. In the absence of any suitable internal disinfection procedure, the machine may be put back into routine use once a

large number of uninfected samples, or an equivalent volume of a fluid such as saline, have been processed through it. As a suggestion, at least 20 uninfected samples should be passed through the machine prior to its return to routine use.

Potentially contaminated drainage from machines used to process blood, serum or other body fluids must either pass into the sewerage system via a sealed drainage system or it must pass into a container via a sealed drainage system. In the latter case the container should contain sufficient sodium hypochlorite solution to produce a final concentration of at least 1% (10000 ppm) when the container is full. The container can be emptied into the sewerage system provided the waste has had a minimum contact time with the hypochlorite of 10 minutes.

9. The specimen should be inactivated if this is possible without compromising the accuracy of testing (see appendix C).

i. Heating at 60 °C for 60 minutes to inactivate serum samples or other body fluids has been recommended by the Centers for Disease Control and Prevention, but is not recommended for nucleic acid tests, as test sensitivity may be markedly reduced. Pre-treatment of EDTA blood is achieved by the lysis procedure used for nucleic acid extraction, e.g. TRIZOL® reagent (guanidinium thiocyanate).

ii. For biochemical testing, heating does not significantly affect estimations of sodium, potassium, magnesium, urea, urate, creatinine, bilirubin, glucose and C-reactive protein. Other tests showed some variation, while enzymes such as alkaline phosphatase, alanine aminotransferase, gamma-glutamyl transferase and creatinine kinase are affected by heat inactivation. This temperature is liable to coagulate IgG and invalidate serological tests. Based on experience with other viruses, laboratories may elect to use 57 °C for 60 minutes to provide sufficient viral inactivation. Serological tests can be performed following this treatment.

iii. Treatment of serum or other body fluids with 10 mL of 10% Triton X-100 per ml of fluid for 1 hour is recommended by the World Health Organization to reduce titres of virus in serum. As this is a detergent, it may affect the performance of tests, particularly where preservation of cells is important.

iv. Air-dried thick blood films should be fixed in 10% buffered formalin for 15 minutes. After formalin treatment, films should be washed 3 times in distilled water at pH 7.0 and then stained.

v. Thin films should be fixed in methanol for 5 minutes and then in 10% buffered formalin for 15 minutes OR fixed in methanol for 30 minutes followed by dry heat at 95 °C for 1 hour. After formalin treatment, films should be washed 3 times in distilled water at pH 7.0 and then stained.

vi. Tissue samples for histology may be fixed in 10% buffered formalin or 2.5% glutaraldehyde for sufficient time to fully penetrate the specimen. This must be verified by slicing through the thickest section of the sample.

vii. The lysis procedure used for nucleic acid detection is adequate to inactivate other specimens for nucleic acid testing, including fluids, stools and swabs. Tissues may be fixed in 10% buffered formalin or other tissue fixatives that are suitable for use prior to nucleic acid amplification.

viii. Specimens for immunofluorescent antigen detection are inactivated following fixation. Acetone 85–100%, glutaraldehyde 1% or greater, or 10% buffered formalin for 15 minutes are satisfactory for inactivating the virus.

SAMPLES THAT HAVE BEEN INACTIVATED CAN BE PROCESSED AS ROUTINE DIAGNOSTIC SAMPLES USING STANDARD LABORATORY PRECAUTIONS.

Advice on which test may be reliably performed on inactivated sample should be obtained from the laboratory attached to the designated isolation hospital.

IT IS STRONGLY RECOMMENDED THAT SPECIMENS THAT CANNOT BE ADEQUATELY INACTIVATED BE SENT TO THE LABORATORY ASSOCIATED WITH THE DESIGNATED ISOLATION HOSPITAL FOR TESTING AIMED AT PREVENTING TRANSMISSION OF BLOOD-BORNE PATHOGENS.

10. Abundant supplies of disinfectants must be available, i.e. 0.5% (5000 ppm) sodium hypochlorite (10% bleach), 70% alcohol and 1% glutaraldehyde. These should be prepared fresh daily. Disinfection material, eye wash solution and hand wash solutions (Betadine and chlorhexidine in alcohol) must be available.
11. Centrifuging of samples must be done in a centrifuge with a sealed rotor or with sealed buckets which are only opened in a class 1, 2 or 3 BSC.
12. After processing, the samples that are to be sent to other areas within the same laboratory should be externally cleaned with 0.5% (5000 ppm) sodium hypochlorite solution and repackaged like the original sample. Specimens should be clearly marked as “Inactivated no VHF Risk” or as “Not Inactivated—VHF Risk”. Staff from the receiving area should give all samples directly to the persons performing the assay. Sample must not be left unattended.
13. Following testing, samples that have not been inactivated must be forwarded to the laboratory of the designated isolation hospital for storage or disposal. They should be packaged in a manner equivalent to current IATA requirements and should be clearly marked for disposal if testing is complete. If storage is required, then the storage instructions must be clearly marked on the outside of the container, or attached to the external container with plastic adhesive tape. The package must remain under the direct control of a responsible individual at all times.
14. A record of all reagents and materials used must be kept for charging purposes. Please note that recompense for costs related to management of these patients requires the prior approval of the Director of Human Quarantine at the Department of Health.

Management of Accidents

1. Laboratory personnel accidentally exposed to potentially infected material (e.g. through injections, cuts or abrasions on the hands) should immediately wash the infected part with soap and water and apply a disinfectant solution e.g. 70% (w/v) alcohol or Betadine. If infected material is accidentally splashed into the eyes, wash thoroughly with eye wash solution provided. Do not use any other disinfectants. In case of heavy contamination of clothing, the contaminated clothing must be discarded in the laboratory and the person should shower immediately. An incident report must be completed. The person should be considered

as a high-risk contact and given post-exposure ribavirin (if indicated) and placed under surveillance (see appendix G). Notify the clinical microbiologist/virologist and the relevant Safety Officer.

2. Accidental spills of potentially contaminated material should be covered with an incontinence pad saturated with 1% (10000 ppm) sodium hypochlorite, left to soak 30 minutes, and then wiped up with absorbent material soaked in 1% (10000 ppm) sodium hypochlorite solution. The waste should be placed in a biohazard bag. With the help of an assistant, this bag should be placed inside another biohazard bag and sealed with tape for disposal. If accidental spills of potentially contaminated material result in aerosol formation (e.g. major spills outside a class 1, 2 or 3 BSC), evacuate the laboratory for 1 hour then proceed as.

MORE DETAILED ADVICE ON HANDLING OF THESE SAMPLES AND LABORATORY SAFETY CAN BE OBTAINED FROM THE LABORATORY ASSOCIATED WITH THE DESIGNATED ISOLATION HOSPITAL.