Technical Report Series

Laboratory Procedures and Precautions for Samples Collected from Patients with Viral Haemorrhagic Fevers

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 B
Guidelines for laboratories that are associated with a designated isolation hospital

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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.

General instructions

A) Specimens

1. Ebola virus and Marburg virus are classified as Tier 1 Security Sensitive Biological Agent (SSBA). Specimens should be handled and transported in accordance with current regulatory requirements.

2. Testing should be kept to the minimum necessary for the management of the patient. This should be discussed with the appropriate specialist for each laboratory area.

3. Wherever possible specimens should be collected at predetermined 'routine' times to allow the laboratory to plan for specimen processing. Specimens must be collected and transported to the laboratory with appropriate precautions (see appendix A). Laboratory staff must be notified before specimens are sent.

4. The sealed specimen must be transported directly to the Designated Receiving Area (DRA – see appendix B) in the laboratory and laboratory staff must directly receive the specimen. The specimen must not be left unattended.

5. Specimens must not be sent by any automatic transport system (e.g. pneumatic air tube system) and must not to be processed in the routine specimen reception area.

6. Determine the analytical technique of the test requested, and if the test is carried out in an open or closed system.

- Tests done in closed systems (e.g. automated analysers) are considered to pose minimal risk to laboratory staff, similar to the processing of specimens collected from patients with other blood-borne viruses such as Hepatitis B, Hepatitis C and HIV. Provided there is no potential for aerosol generation, manual uncapping of blood tubes, proper disposal of waste and that the machine can be decontaminated after each use, non-inactivated specimens may be processed in automated analysers using standard laboratory precautions.
- Open system tests, including manual uncapping of blood tubes and centrifugation in a non-sealed centrifuge, should be avoided on non-inactivated samples.
- Protocols for machine decontamination, maintenance and management of spillages and waste disposal must be in place and followed.

7. Samples may be inactivated (see appendix C) before they are tested, provided the process of inactivation does not invalidate the test. This must be done within the DRA. Samples that have been inactivated may be processed as routine samples using standard laboratory precautions.

8. Point-of-care (POC) devices may be suitable for use in patients with suspected or confirmed VHF. POC testing is available for basic haematology, coagulation, arterial or venous gases and biochemistry. POC devices have been previously used in managing patients with Ebola virus disease in a developed country, but the accuracy of these devices compared to standard analysers was not reported (Hill *et al*).

9. Following testing, samples that have not been inactivated must be returned to the DRA for storage or disposal. They should be packaged in the same way as the initial sample (see appendix A) and should be clearly marked on the outside for disposal if testing has been completed. 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.

10. Waste from automated analysers is not considered to pose a significant risk because of the small sample volumes and their dilution in processing, and requires no special waste disposal precautions.

B) Designated Receiving Area (appendix B)

1. This area is responsible for the initial processing of all samples, and for the storage and disposal of specimens and waste.

2. A senior member of the microbiology or virology staff should be designated to manage this area.

3. The special requirements for this area are contained in appendix B.

C) Other Laboratory Areas – Precautions for Handling Non-inactivated Samples

1. Each laboratory carrying out tests on non-inactivated samples must have a senior staff member who coordinates the testing and liaises with other laboratories and the DRA.

2. Wherever possible, tests should be carried out at specified times which are coordinated with the specimen collection and processing times. For example, laboratories may elect to carry out tests on these samples at lunchtime when the area is quieter and morning samples have been processed through the DRA.

3. Personnel involved in handling laboratory specimens must be kept to a minimum. It is preferred that senior staff are designated to process these samples. All nonessential staff must vacate the immediate area during testing in case of spills.

4. Laboratory staff dealing with these specimens must wear appropriate personal protective equipment (PPE) and follow other precautions as specified in appendix D.

5. 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. Further detail is included in appendix D.

6. Specimen containers must only be opened in a class 1, 2, or 3 biological safety cabinet (BSC). Every effort should be made to avoid creating an aerosol or splash. PROCEDURES THAT ARE LIKELY TO PRODUCE AEROSOLS OR SPLASHING SHOULD ONLY BE CARRIED OUT IF ABSOLUTELY NECESSARY. THEY MUST NEVER BE PERFORMED OUTSIDE A CLASS 1, 2 OR 3 BSC. For centrifuging, the sample must be in a sealed centrifuge bucket or rotor.

7. Facilities or equipment used to handle these specimens must be cleaned and/or decontaminated according to the instructions in appendix E.

8. The laboratory door must be closed during testing and have a sign affixed stating: "TESTING OF VHF SAMPLES. DO NOT ENTER"

9. For disposal of waste refer to appendix F.

D) Specific Instructions for Speciality Areas

Testing algorithms incorporating VHF and non-VHF tests using routine automated analyzers and/or POC devices may be devised for patients with suspected or confirmed VHF. Although the range of tests available in POC devices are limited compared to routine analyzers and there is no published data on their use in patients with VHF, they are simple tests that are relatively easy to perform and test kits and cartridges may be easily disposed.

Following confirmation of VHF, more specific haematology and biochemistry testing may be necessary in the management of patients. However, some laboratories and clinicians may choose to continue using POC devices. A decision will also need to be made as to whether POC devices are placed at the bedside, or in the laboratory. Quality assurance is paramount irrespective of the location of POC devices.

1) Microbiology

Microbial testing should not be performed on suspected VHF cases until it has been discussed with the medical microbiologist. Microbial tests include VHF testing *per se*, and testing of other pathogens.

The National High Security Quarantine Laboratory (NHSQL) at the Victorian Infectious Diseases Reference Laboratory, Peter Doherty Institute for Infection and Immunity in Parkville, Melbourne is the Australian Government Department of Health national reference laboratory for the diagnosis of VHF. Information on VHF testing at NHSQL may be found at the Victorian Infectious Diseases Reference Laboratory website. VHF testing is also available at CIDMLS, ICPMR, Westmead in NSW and Forensic and Scientific Services, Coopers Plains in QLD.

Nucleic acid testing is the preferred test to confirm or exclude the diagnosis of VHF. Where possible, 3 × 4 mL (total of 12 mL) of blood should be collected into EDTA tubes. Following VHF testing in NSW and QLD, the extra tubes may be sent to the NHSQL and/or CDC for confirmation of VHF. Malaria testing (see below) can also be performed using this sample.

1.1 Malaria

1.1.1 Exclusion of malaria is an essential part of the initial investigation of a patient suspected to have VHF. This can be done using malaria rapid diagnostic tests (RDTs) and/or blood films. If malaria tests are positive, other tests needed for patient management can be handled in the routine manner provided clinicians managing the patient are happy that this explains the illness. However, it should be remembered that malaria parasites might be found in the blood of patients with other diseases.

1.1.2 Malaria RDTs can detect different malarial species with varying sensitivities and specificities using histidine-rich protein 2 (HRP2) and/or plasmodial lactate dehydrogenase (pLDH) as antigens. The accuracy of RDTs in diagnosing uncomplicated *P. falciparum* infection is equal or superior to routine, but not expert microscopy. Diagnostic performance of RDTs in the detection of non-falciparum malarial species is poor. For the detection of *P.*

falciparum, the sensitivity and specificity of RDTs is ~95% for HRP2 and 93% and 99% respectively for pLDH (Abba *et al*). Note that HRP-2 antigen can persist even after effective treatment.

1.1.3 Malaria films may also need to be performed in cases where the malaria RDT is negative. Thick and thin blood films should be prepared (see appendix C) in the DRA by an experienced person and rendered safe before being released for staining and examination. After preparation, blood films may be examined using the light microscope in a routine manner.

1.2 Bacteriology

Bacteriology testing should not be performed on suspected VHF cases until it has been discussed with the medical microbiologist, or after exclusion of VHF.

1.2.1 Routine diagnostic bacteriology of blood, urine, sputum, faeces, CSF, wound or genital specimens may be indicated. Specimens must be plated using disposable instruments in a level 1, 2 or 3 BSC in the DRA. All primary bacterial cultures should be sealed and incubated in a CO_2 incubator in the DRA.

1.2.2 Cultures requiring other atmospheric conditions should be sealed and gassed in jars, and incubated in the bacteriology laboratory but only re-opened and read in the DRA.

1.2.3 Blood cultures should not be processed by automated instruments where automatic venting of the culture is required, but should be subcultured to agar plates as required. Fully enclosed automated blood culture systems may be used without special precautions. All subculturing must be done in the DRA. All secondary cultures can be handled in the routine laboratory.

1.3 Virology

Virology testing should not be performed on suspected VHF cases until it has been discussed with the medical microbiologist, or after exclusion of VHF.

1.3.1 Immunofluorescence for antigen detection can be performed once the slides have been fixed in DRA (see appendix C).

1.3.2 Cell cultures must not be performed on samples from suspected VHF cases.

1.3.3 Nucleic acid tests may be performed routinely on samples that have been inactivated. Refer to appendix C for inactivation methods.

1.4 Serology

Serology testing should not be performed on suspected VHF cases until it has been discussed with the medical microbiologist, or after exclusion of VHF.

1.4.1 Serology testing may be done on automated analysers provided that it is a closed system, without manual uncapping of the blood tubes and that an adequate procedure for disinfection of the analysers is carried out after sample processing and before the analyser's scheduled maintenance.

1.4.2 If the serology test requested is an open system test or requires manual uncapping of the blood tubes, then these tests must be done on inactivated sera (see appendix C). However, inactivation of sera using heat, formalin, Triton X-100 or β-propriolactone may affect the performance of the test. If testing heat-inactivated specimens, laboratory and manufacturers' controls should also be tested. Provided the control results are satisfactory, the results may be released as provisional results. If VHF is subsequently excluded, the tests should be repeated on non-inactivated specimens.

1.4.3 Disinfection and decontamination procedures validated as effective against blood-borne viruses must be in place.

2) Haematology

Haematological testing should not be performed on suspected VHF cases until it has been discussed with the clinical haematologist. This may be performed on non-inactivated specimens using automated analysers (see above). Where available, limited haematology testing can be performed using POC devices. Most existing POC devices measuring clotting parameters were developed to monitor oral anticoagulation treatment. However, note that current evidence at the time of writing suggests that in the context of acute haemorrhage, there may be a lack of agreement between POC devices and standard laboratory tests in detecting coagulopathy (Gauss *et al*). At present, there is limited utility of non-invasive, continuous haemoglobin monitoring using spectrophotometry-based devices in patients at risk for haemorrhage.

The following tests are available in a POC format; examples of the devices available include:-

- Haemoglobin (HemoCue® Hb Analyser, Abbott i-STAT®)
- Haematocrit (Abbott i-STAT®)
- PT/INR and aPTT (Hemochron® SIGNATURE +)
- PT/INR (Abbott i-STAT®, Roche CoaguChek® XS Pro)

- Activated clotting time (Abbott i-STAT®)
- Platelets (ROTEM® *platelet*)

2.1 Haematological specimens can be processed in a Coulter counter provided that it does not require manual uncapping of the blood tube and that there is proper disposal of waste fluids (see appendix F). The Coulter counter can be internally cleaned after use with several cycles of 0.5% (5000 ppm) sodium hypochlorite solution and the external surfaces wiped over with the same solution. Alternative decontamination procedures recommended by the instrument's manufacturer must be verified to be adequate to inactivate the agents of VHF. Samples with insufficient volume for testing should not be processed.

2.2 If a blood film is necessary following processing in the Coulter counter, the sample must be returned to the DRA for the film to be made (see appendix C). Hold all films until the patient is discharged for comparison, if required.

2.3 Coagulation studies can be performed using a machine that does not require removal of the top of the blood collection tube and where there is proper disposal of waste fluids (see appendix F) and the machine can be decontaminated after use (see appendix E). Otherwise, a suitably experienced individual in the DRA must perform coagulation studies using manual methods.

2.4 Cross-matching of blood or blood products for transfusion is not recommended due to the complexity and hazard to the technician. O negative packed red blood cells, low haemolysin group O platelets and/or group AB fresh frozen plasma will be used if required.

2.5 Attempts to inactivate blood specimens for haematological testing using β-propiolactone or 3% acetic acid have been tried. β-propiolactone causes an unacceptable degree of haemolysis resulting in inaccurate complete blood count results from a Coulter counter. 3% acetic acid treatment alters the light-scatter characteristics of the white blood cells to an extent where the differential white blood cell count is compromised.

3) Biochemistry and arterial blood gases

Biochemistry and arterial blood gas testing of VHF specimens should not be performed on suspected or confirmed VHF cases until it has been discussed with the clinical chemist.

3.1 Where available, limited biochemistry testing may be performed on POC devices. The following tests are available in the POC format; such examples of the instruments available include:-

- Sodium, potassium, calcium, glucose, creatinine, urea, lactate, calcium, chloride, CO_2 (Abbott i-STAT®)
- pH, pCO_2, pO_2, HCO_3, base excess, SaO_2 (Abbott i-STAT®)

Previous evaluations of the Abbott i-STAT® have shown good correlation with laboratory measurements of pH, pO_2 and pCO_2 in ventilated patients in the intensive care unit (Thomas *et al*); haematocrit, sodium, potassium and glucose in patients undergoing cardiopulmonary bypass (Connelly *et al*); and creatinine values to estimate glomerular filtration rates (Naugler *et al*).

3.2 In patients with confirmed VHF, a decision will need to be made if the available POC tests are adequate for the management of patients.

3.3. In cases where there is no POC test or if POC was deemed to be inadequate, non-inactivated specimens can be processed in automated analysers that do not require removal of the top of the blood collection tube, provided that the machine can be decontaminated after use (see appendix E) and there is proper disposal of waste fluids (see appendix F). Otherwise, a suitably experienced individual in the DRA must perform tests using manual methods. Routine cleaning and disinfecting procedures can be used for automated analysers as recommended by the manufacturer.

3.4 Heat inactivation of specimens (56 °C for 30 minutes or 60 °C for 60 minutes) can affect the results of some biochemical tests, including electrolytes, liver function tests and cardiac enzymes (Bhagat *et al*) (see appendix C). If heat-inactivated specimens are tested, laboratory and manufacturers' controls should also be tested. Provided the control results are satisfactory, the results may be released as provisional results. If VHF is subsequently excluded, the tests should be repeated on non-inactivated specimens.

4) Immunology

Testing should not be performed on suspected VHF cases until it has been discussed with the clinical immunologist.

4.1 Similar to testing of samples in the disciplines outlined above, testing of non-inactivated specimens may be performed on automated analyzers.

4.2 Manual testing of non-inactivated specimens should be avoided. In such instances, testing may be performed on heat-inactivated specimens, noting that the use of heat-inactivated specimens may affect the results of tests. Laboratory and manufacturers' control should be tested, and provided the control results are satisfactory, provisional results may be released. If VHF is subsequently excluded the tests should be repeated on non-inactivated serum.

4.3 Immunofluorescence tests may be performed following fixation in 85-100% acetone.

4.4 Nucleic acid detection assays can be performed on samples that have been inactivated (refer to appendix C).

4.5 Complement assays and tests for cellular immunity cannot be performed.

Tests cannot be performed on unfixed tissue.

5) Tissue Pathology

Testing should not be performed on suspected VHF cases until it has been discussed with the histopathologist.

5.1. Specimens will only be processed following adequate fixation in 10% buffered formalin or 2.5% glutaraldehyde in DRA. Adequate fixation must be determined following slicing of the tissue in the DRA by a suitably experienced individual.

5.2 Frozen sections must not be performed.

5.3 Autopsies must not be performed.

6) Nucleic Acid Detection

6.1 These tests may be performed routinely on samples that have been inactivated. Refer to appendix C for inactivation methods.

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Appendix A

Specimen Collection Transport and Storage

1. During collection every effort must be taken to avoid external contamination of the specimen tube or container. An assistant should receive the labelled specimen into the plastic bag at the bedside, which is then sealed and placed into a clear hard plastic container. The outer container must have sufficient absorbent material to contain a spill. The external surface of the outer container must be wiped or sprayed with 0.5% (5000 ppm) sodium hypochlorite and allowed to dry. The request form should be attached to the exterior using plastic tape. The request form should never be placed in the same container as the specimen, nor should it be attached with pins or staples. Laboratory staff must be notified when specimens are sent.

2. The sealed specimen should be transported directly to the Designated Receiving Area (DRA) in the laboratory and laboratory staff must directly receive the specimen. The specimen must not be left unattended.
Specimens must not be sent by any automatic transport system (e.g. pneumatic air tube system) and must not to be processed in the routine specimen reception area.
The outer containers may be reused provided there has been no spillage. They must be autoclaved or decontaminated in 1% glutaraldehyde for 20 minutes within the DRA prior to release.

3. If inactivation of the sample is possible, it must be done within the DRA prior to the release of the samples into the routine laboratory areas. Refer to appendix C for suitable methods for inactivation.

4. After processing, the primary container for samples to be sent to other laboratory areas for testing 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"**. All samples should be given directly to the persons performing the assay by staff from the DRA receiving area. Samples must not be left unattended.

5. ***Samples which have been inactivated and externally decontaminated may be processed as routine diagnostic samples using standard (BSL 2) laboratory precautions***

6. Following testing, samples that have not been inactivated must be returned to the DRA receiving area for storage or disposal. They should be packaged in the same way as the initial sample and should be clearly marked on the outside 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.

Appendix B

Minimum Requirements for the Designate Receiving Area

1. The room must be physically separated from other areas by a door.

2. The room must be able to be sealed for decontamination.

3. The room must contain at least one Class 1, 2 or 3 biosafety cabinet, a laboratory sink, a hand washing sink, a refrigerator and a –20 °C freezer. A water bath or heating block will be required for inactivation of serum. Other equipment required will depend upon the types of specimens, processing and testing to be undertaken in the DRA.

4. All equipment for specimen processing must either be located within a biosafety cabinet or employ a sealed system. Other equipment should be removed if possible.

5. Equipment used in the DRA must be dedicated to that room and cannot be moved to other areas without undergoing decontamination.

6. There must be sufficient space for storage of samples at 4 °C and –20 °C. Storage of samples outside this area is to be avoided. If samples must stored at lower temperature and/or cannot be accommodated in this area, then they must be repackaged like the original sample, marked clearly and then placed in a designated place (e.g. a –70 °C freezer). The storage place must be locked and the key held by the staff member responsible for the DRA receiving laboratory.

7. The laboratory door must be closed at all times and have a sign affixed stating: “TESTING OF VHF SAMPLES. DO NOT ENTER”

8. It is preferable that the laboratory has its own autoclave or one located nearby.

9. It is preferable that the laboratory has an attached shower and change room.

10. Personnel involved in handling laboratory specimens must be kept to a minimum. It is preferred that senior staff are designated to process these samples.

11. Staff working in this area must have sufficient skills to carry out the processing of the likely specimen types that will be received. It is the responsibility of the final testing laboratory to ensure that adequate instructions are provided, or to supply a suitably skilled person. All samples must be handled in a class 1, 2 or 3 BSC.

12. Centrifuging of samples must be done in a centrifuge with sealed buckets, which are only opened in a class 1, 2 or 3 BSC.

13. The laboratory must be locked when not in use and the key held by the staff member responsible for that area. Other designated personnel (e.g. security staff) may hold spare keys.

14. Laboratory staff dealing with specimens must wear full protective clothing consisting of gloves, impervious long-sleeve gowns, shoe covers, duckbill masks and a full-face visor. Disposable overalls must be worn under the impervious long-sleeve gowns. The full-face visor is unnecessary if working in a class 1, 2 or 3 Biological Safety Cabinet (BSC). Every

effort should be made to avoid creating an aerosol or splash. All other requirements in appendices D, E, and F must be met.

15. On leaving the laboratory
15.1 Gloves, mask, gown, cap and overalls must be placed in a biohazard bag in the laboratory and immediately sealed for disposal. If any of the clothing was contaminated by potentially infectious material then, with the help of an assistant, the first bag should be placed inside another biohazard bag and sealed with tape. The outside of the bag must be wiped over with 0.5% (5000 ppm) sodium hypochlorite before leaving the laboratory.
15.2 Face visors should be immersed in 0.5% (5000 ppm) sodium hypochlorite 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.
15.3 Contaminated overalls must be discarded before leaving the laboratory as in 15.1.
15.4 Hands must be washed after leaving the room with Betadine (if iodine-allergic, chlorhexidine) under running water.

16. Potentially contaminated fluids must either be autoclaved prior to leaving the area or they must be discarded into a container that contains sufficient sodium hypochlorite to produce a final concentration of at least 1% (10000 ppm) sodium hypochlorite when the container is full. The container can be emptied into the sewerage system provided there has been at least 10 minutes contact time with the hypochlorite.

17. Prior to leaving the DRA, all waste must either be
17.1 sterilised in an autoclave
17.2 double bagged and the outer bag wiped over with 0.5% (5000 ppm) sodium hypochlorite
17.3 placed in a puncture proof container (for sharps) which must then be placed in an outer bag that is then wiped over with 0.5% (5000 ppm) sodium hypochlorite. These must then be placed in a rigid container for transport to the incinerator.

Appendix C

Procedure for Inactivation of Samples

The following methods are suitable for producing acceptable reduction of infectivity in order to allow processing of samples using standard (BSL 2) laboratory precautions.

1. 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).

2. 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.

3. 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.

4. 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.

5. 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.

6. 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.

7. 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.

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

Appendix D

Staff Protection and Management of Exposure to Potentially Infectious Material

1. Full protective clothing consisting of double gloves, impervious long-sleeve gowns, shoe covers, N95/P2 masks and disposable full-face visors must be worn. Theatre overalls or disposable overalls should be used under the impervious long-sleeve gowns. The full-face visor is unnecessary if working in a class 1, 2 or 3 Biological Safety Cabinet (BSC).

2. A written record of all personnel involved in laboratory testing must be kept to record dates, times and analyses performed for each person. 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 in each area. A record of all reagents and materials used must be kept for charging purposes.

3. 10 mL of clotted blood must be collected from staff handling specimens from suspected VHF patients which will be stored in Virology for baseline serology should it be required.

4. 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.

5. On leaving the laboratory or work area

a) gloves, impervious long-sleeve gowns, shoe covers, N95/P2 masks and the full-face visor must be placed in a biohazard bag in the laboratory and immediately sealed for disposal. The bag must be returned to the DRA receiving area for disposal. If any of the clothing was contaminated by potentially infectious material then, with the help of an assistant, the first bag should be placed inside another biohazard bag, sealed with tape and returned to the DRA for disposal. Uncontaminated overalls can be sent for laundering as usual.
b) following any episodes of contamination, face visors must be immersed in 0.5% (5000 ppm) sodium hypochlorite for 10 minutes, washed and dried before re-use.
c) contaminated overalls must be discarded before leaving the laboratory as in (a).
d) hands must be washed on leaving the room with Betadine (if iodine-allergic, chlorhexidine in alcohol) under running water.

6. No pregnant, or immunocompromised staff should work with specimens from patients with suspected VHF.

Appendix E

Cleaning and Decontamination of Laboratory Facilities and Equipment

1. Abundant supplies of disinfectants must be available, i.e. 0.5% (5000 ppm) sodium hypochlorite, 70% (w/v) alcohol and 1% glutaraldehyde. These should be prepared fresh daily. Disinfectants, eye wash solution and handwash solutions (Betadine and chlorhexidine in alcohol) must be available.

2. Glutaraldehyde is potentially toxic and must either be used in a sealed container or the room must be vacated following use and remain empty until the odour has dissipated. Local Occupational Health and Safety requirements must be met.

3. 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% 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.

4. 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 in (3).

5. BSC's must be cleaned after spills, and at the completion of work with potential VHF samples. Spills must be dealt with as in (3) above. The BSC should then be wiped over with 1% glutaraldehyde or 0.5% (5000 ppm) sodium hypochlorite solution left for 10 minutes. Hypochlorite will need to be wiped off to reduce corrosion. If glutaraldehyde has been used, the room will need to be vacated until the odour has dissipated. Local Occupational Health and Safety requirements must be met.

6. Centrifuge buckets or rotors must be autoclaved or immersed in 1% glutaraldehyde (in a sealed container) for 10 minutes.

7. Automated machinery should be decontaminated with 0.5% (5000 ppm) sodium hypochlorite for several cycles and the external surfaces wiped over with 0.5% (5000 ppm) sodium hypochlorite. 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 blood-borne viruses such as hepatitis B, hepatitis C or HIV, 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, has 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.

8. Racks used to carry specimens must be placed in a plastic bag after use and sealed. This bag should be placed inside another bag and the outside must be wiped over with 0.5% sodium hypochlorite. The bag should be clearly marked with the nature of the contents and sent to the DRA.

Appendix F

Waste Disposal

1. All patient specimens, materials used for culturing patient samples and all contaminated glassware or equipment must be placed in puncture proof containers. The container must be placed in a plastic bag, sealed and the outside must be wiped over with 0.5% (5000 ppm) sodium hypochlorite solution. The bag should be clearly marked with the nature of the contents and indicate that they are for disposal and is to be returned to the DRA.

2. Reusable items that cannot be sterilised in an autoclave must be placed in puncture proof containers. The container must be placed in a plastic bag, sealed and the outside must be wiped over with 0.5% (5000 ppm) sodium hypochlorite solution. The bag should be clearly marked with the nature of the contents and indicate that they are for re-use and is to be returned to the DRA.

3. Disposable sharps must be placed in a puncture-proof approved sharps container. When full, the container must be placed in a plastic bag, sealed and the outside must be wiped over with 0.5% (5000 ppm) sodium hypochlorite solution. The bag should be clearly marked with the nature of the contents and indicate that they are for disposal and is to be returned to the DRA.

4. General laboratory waste must be placed in waterproof bags, then placed in a plastic bag, sealed and the outside must be wiped over with 0.5% (5000 ppm) sodium hypochlorite solution. The bag should be clearly marked with the nature of the contents and indicate that they are for disposal and is to be returned to the DRA.

5. 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 to produce a final concentration of at least 1% 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.

Appendix G

Management of Laboratory Personnel Accidentally Exposed to Potentially Infectious Material

This includes all staff members who have had exposure to blood or body fluids from proven or suspected cases. Significant exposures are needlesticks, contact with mucous membranes (eyes, mouth or nose) or contact with broken skin.

Prophylaxis with ribavirin has been shown to be effective for Lassa fever virus and, based on *in vitro* susceptibility data, it may also be useful for CCHF. There is no evidence that it is likely to be effective for Ebola or Marburg viruses. Therefore its use should be restricted to contacts with cases of undetermined cause, or with proven Lassa fever or CCHF virus. It cannot be used for pregnant women.

1. The incident must be reported and the patient referred urgently to the clinical microbiologist and/or infectious diseases physician.

2. The exposed individual must report daily and have their temperature monitored daily. If the person becomes unwell or develops fever, then they require urgent review by the clinical microbiologist and/or infectious diseases physician.

3. Ribavirin prophylaxis, if appropriate, is given as 500 mg orally qid for 1 week in adults. Alternatively a lower dose of 5 mg/kg tds may be used for 2–3 weeks.