

Communicable Diseases Intelligence



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Laboratory Precautions for Samples Collected from Patients with Suspected Viral Haemorrhagic Fevers



*Laboratory Precautions
for Samples
Collected from Patients
with Suspected Viral
Haemorrhagic Fevers*

Parts A and B

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*Laboratory Precautions
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Part B

Guidelines for Laboratories
that are Associated
with a Designated
Isolation Hospital

Introduction

There are a large number of viruses that cause haemorrhagic fever, but only a few are known to pose a risk of transmission within health care settings. This group of VHF's comprises Lassa fever (LF), Congo-Crimean haemorrhagic fever (CCHF), Ebola virus and Marburg virus infection. 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 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 health care workers within modern health care settings occurred during the Marburg virus outbreaks and possibly from an Ebola case in South Africa, but they are rare. However because of this potential and because these diseases have a high mortality, 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 a 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 the State or Territory VHF Response 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 viral haemorrhagic fevers 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. High standards of routine patient care and specimen handling should offer good protection, however assessment and surveillance of staff who had contact with the patient or samples would be necessary. Fortunately patients with advanced and highly infectious disease are more likely to be clinically recognised and transferred to a designated hospital. Also past Australian experience with suspected cases shows that most are caused by other infectious agents (e.g. malaria, streptococcal pharyngitis, and HIV seroconversion) and none have yet been genuine VHF cases.

Within designated hospitals and laboratories, facilities for the clinical care and diagnosis of cases of viral haemorrhagic fever (VHF) face a number of problems. As suspected cases are rare, the patient care and laboratory facilities are generally used for other purposes, and must be made available for VHF cases at short notice. Also, there are potentially a wide range of tests required for these patients, which cannot all be performed within a specified area and the routine laboratories have to be used. With rare exceptions, laboratories do not have access to biosafety level (BSL) 4 facilities and, usually, do not have BSL 3 facilities. Therefore the precautions to be used must be based on enhancing protection within a BSL 2 environment.

General instructions

A) Specimens

1. 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.
2. Wherever possible specimens should be collected at a 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.
2. 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.
3. Specimens must not be sent by any automatic transport system (e.g. vacuum tubes) and must not to be processed in the routine specimen reception area.
4. Wherever possible samples must be inactivated (see Appendix C) before they are tested. This must be done within the DRA. Samples that have been inactivated may be processed as routine samples using standard (BSL 2) laboratory precautions.
5. 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.

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 will 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 protective clothing 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

1) Haematology

Haematology tests should not be performed on suspected VHF cases until it has been discussed with the Clinical Haematologist.

The following tests are likely to be required

- Thick and thin malaria blood films
 - Haemoglobin
 - Haematocrit and blood film, including WCC and differential counts, and platelet count
 - Coagulation studies
 - Cross-matching of blood cannot be performed safely.
- 1.1. Malaria films are an essential part of the initial investigation of a patient suspected to have a VHF, as malaria is the illness most likely to be confused with a VHF. Other investigations will be performed as clinically indicated. If malaria parasites are demonstrated, other tests needed for patient management can be handled in the routine manner provided the medical team 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. If malaria parasites are not seen then the diagnosis of a VHF must be considered and specimens handled as described above.

Thick and thin blood films should be prepared in the DRA by an experienced person and rendered safe before being released for staining and examination. Air dried films should be fixed in 10% buffered formalin for 15 minutes and thin films should be fixed in methanol for 5 minutes and then in 10% buffered formalin as for thick films. After formalin treatment, films should be washed 3 times in distilled water at pH 7.0 and then stained. Alternatively, thin films can be rendered safe by fixing in methanol for 30 minutes followed by dry heat at 95°C for 1 hour. Blood films may then be examined using the light microscope in a routine manner.
 - 1.2 Haematologic specimens can be processed in a Coulter counter provided that it does not require removal of the top of the blood collection 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% hypochlorite and the external surfaces wiped over with 0.5% hypochlorite. If the manufacturers recommend an alternative decontamination procedure, then it must be verified that it is adequate to inactivate the agents of VHF. Samples with insufficient volume for sampling through the lid cannot be processed.

- 1.3 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. The films can be fixed in methanol for 5 minutes and then in 10% buffered formalin for 15 minutes and released to the routine laboratory. Hold all films until the patient is discharged for comparison, if required.
- 1.4 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.
- 1.5. Cross-matching of blood or blood products for transfusion will NOT be performed owing 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. If testing for red blood cell antibodies is required, heat-inactivated serum may be satisfactory.
- 1.6. Attempts to decontaminate blood specimens with beta-propiolactone or 3% acetic acid have been tried. Beta-propiolactone causes an unacceptable degree of haemolysis resulting in inaccurate FBC results from a Coulter counter. 3% acetic acid treatment alters the light scatter characteristics of the WBC to an extent where the differential WBC is compromised.

2) Biochemistry

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

The following tests are likely to be required

- Sodium, potassium, protein, alkaline phosphatase, amylase, creatinine, glucose, AST/ALT, uric acid (or urea), bilirubin, creatinine kinase.
 - Blood gases
- 2.1. Wherever possible, these tests must be done on inactivated sera. If the method is not validated for this, then the test should be performed with manufacturer's controls and the laboratory controls. Provided the control results are satisfactory, then the results may be utilised as provisional results. If a VHF is subsequently excluded, then tests should be repeated on non-inactivated serum.
 - 2.2. 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 (see Appendix F) and the machine can be decontaminated after use (see Appendix E). Otherwise, a suitably experienced individual in the DRA must perform tests using manual methods.

3) Bacteriology

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

- 1.1. Routine diagnostic bacteriology of CSF, blood, urine, sputum, faeces, genital or wound specimens can be performed. 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 CO² incubator in the DRA.

- 1.2 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.
- 1.3 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 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.

4) Virology

Testing should not be performed on suspected VHF cases until it has been discussed with the Medical Microbiologist/Virologist.

- 4.1. Immunofluorescence for antigen detection can be performed once the slides have been fixed in DRA by one of the methods given in appendix C.
- 4.2 Cell cultures must not be performed on samples from suspected VHF cases.
- 4.3 The NHSQL at North Melbourne in Melbourne is the designated Australian laboratory for performing tests for diagnosis of a suspected VHF. Testing must not be carried within the local or state reference laboratory. Information on the process for requesting assistance from the NHSQL may be found in the *Guidelines for the Management of Human Quarantine Disease in Australia*.

5) Serology

Testing should not be performed on suspected VHF cases until it has been discussed with the Medical Microbiologist/Serologist.

- 5.1 Wherever possible, these tests must be done on heat-inactivated sera. If the method is not validated for this, then the test should be performed with manufacturer's controls and the laboratory controls. Provided the control results are satisfactory, then the results may be utilised as provisional results. If a VHF is subsequently excluded, then tests should be repeated on non-inactivated serum.
- 5.2 If testing of non-inactivated samples is absolutely necessary, automated machinery must not be used. All processes up to and including the wash step following incubation with the patient's serum (or other fluid) must be performed in the DRA. After that the test may be completed in the routine laboratory.

6) Immunology

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

- 6.1 Autoantibody assays should be performed on heat-inactivated serum, using the manufacturer's controls and the laboratory controls. Provided the control results are satisfactory, then the results may be utilised as provisional results. If a VHF is subsequently excluded, then tests should be repeated on non-inactivated serum.
- 6.2 Immunofluorescence tests may be performed following fixation in 85-100% acetone.
- 6.3 If testing of non-inactivated samples is absolutely necessary, automated machinery must not be used. All steps that include patient material must be performed in the DRA. For serum or other fluids, once the initial wash has been performed the test may be completed in the routine laboratory.
- 6.4 Nucleic acid detection assays can be performed on samples that have been inactivated (refer to appendix C).

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

Tests cannot be performed on unfixed tissue.

7) Tissue Pathology

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

7.1. 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.

7.2 Frozen sections must not be performed.

7.3 Autopsies must not be performed.

8) Nucleic Acid Detection

8.1 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% 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. Vacuum tubes) and must not 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% 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 Designated 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 be 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% hypochlorite before leaving the laboratory.
 - 15.2 face visors should be immersed in 0.5% 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% 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 autoclaved
 - 17.2 double bagged and the outer bag wiped over with 0.5% 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% 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 for serum samples or other body fluids has been recommended by the Centers for Disease Control. Recent work (reference 1) has shown that heating does not significantly affect estimations of sodium, potassium, magnesium, urea, urate, creatinine, bilirubin, glucose and C-reactive protein. Other test showed some variation, while enzymes such as alkaline phosphatase, alanine aminotransferase, gamma-glutamyl transferase and creatinine kinase were inactivated.

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.

2. 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.
3. 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.
4. 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.
5. 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.
6. Specimens for nucleic acid amplification may be inactivated by heat treatment at 60°C for 60 minutes. Swabs will be satisfactorily inactivated once they have been treated with the lysis reagent. Tissues may be fixed in 10% buffered formalin or other tissue fixatives that are suitable for use prior to nucleic acid amplification.
7. Specimens for immunofluorescent antigen detection are inactivated following fixation. Acetone 85-100%, glutaraldehyde 1% or greater, or 10% 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 consists of gloves, impervious long-sleeve gowns, shoe covers, duckbill masks and a full-face visor 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 mls 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 or in charge and the relevant Safety Officer.
5. On leaving the laboratory or work area
 - a) gloves, mask, gown and cap 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) at the end of each shift, or following any episodes of contamination, face visors must be immersed in 0.5% 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 may 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% sodium hypochlorite (10% bleach), 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% 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% hypochlorite which is left 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% hypochlorite for several cycles and the external surfaces wiped over with 0.5% 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 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.
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% sodium hypochlorite. 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 autoclaved 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% sodium hypochlorite. 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% sodium hypochlorite. 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% sodium hypochlorite. 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 or CCHF. It cannot be used for pregnant women.

1. The incident must be reported and the patient referred urgently to the Clinical Microbiologist 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 or Infectious Diseases Physician.
3. Ribavirin prophylaxis, if appropriate, is given as 500mg orally qid for 1 week for adults. Alternatively a lower dose of 5 mg/kg tds may be used for 2-3 weeks.