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GUIDELINES FOR THE SAFE HANDLING OF LABORATORY SPECIMENS FROM CASES OF VIRAL HAEMORRHAGIC FEVER

Introduction

1. This document provides guidance on the risk assessment and handling of laboratory specimens from suspected cases of viral haemorrhagic fevers (VHF). The risk assessment should be based on the instrumentation used, sample requirement, potential for aerosolization, test procedure and other operational considerations. The laboratory should implement measures to adequately manage all the risks identified in the setting of their facilities. Universally applied bloodborne pathogen precautions should be part of the processing steps of diagnostic laboratories.

2. VHF refers to a group of illnesses that are caused by several distinct families of viruses. While some types of haemorrhagic fever viruses can cause relatively mild illnesses, we refer here to those which can cause severe, life-threatening disease. The main agents of concern are Ebola virus, Marburg virus, Crimean-Congo Haemorrhagic Fever (CCHF) virus and Lassa fever virus (i.e. the “exotic” VHF, excluding dengue).

3. The main routes of transmission of VHF infection are **direct contact** (through broken skin or mucous membrane) with the blood or body fluids of infected persons, and **indirect contact** with the environment or objects contaminated with infected blood or body fluids, e.g. contaminated needles and syringes.

4. Specimens, especially blood specimens, from patients with VHF are potentially hazardous to laboratory staff as blood and body fluids may contain high concentrations of the viruses. Precautions should be taken in particular with regards to sharps and needles.

Management of laboratory specimens from patients with suspected VHF

1. Testing should be kept to the minimum necessary for diagnostic evaluation and management of the patient.

2. There should be a system in place for the attending physician to notify the laboratory supervisor before specimens are sent for analysis.

3. Specimen Transport from Ward to the Laboratory

   3.1 Specimens must be collected and hand-delivered from ward to the laboratory with appropriate precautions. Automated transport systems e.g. pneumatic tubes, should not be used. Specimens should be clearly tagged as "VHF Risk" or equivalent (Refer to Annex A for packaging instructions).

   3.2 The specimen should be directly received by designated laboratory staff and must not be left unattended. If testing is not immediate, the specimen should be stored in a safe and secure place (preferable fridge or freezer) with controlled access. Storage and handling instructions should be clearly marked on the outside of the specimen packaging.

   3.3 A senior member of the laboratory staff shall be responsible for looking after the specimens and ensuring safe handling, test performance and disposal. Proper hand-over to another senior member of staff should be done when going off duty.

4. The laboratory should have a designated receiving area (DRA) or cabinet for the initial processing of all specimens from suspect cases, and for the storage and safe disposal of specimens and waste. Waste should be segregated from general laboratory waste. The DRA must be decontaminated in the event of an environmental spill. This area should be managed by a senior member of the laboratory staff.

5. Specimens may be handled in a BSL-2 laboratory with additional precautions, which include the following:

   5.1 Area segregated from the rest of the laboratory and can be decontaminated.

   5.2 Restricted personnel access.

   5.3 Personal protective equipment should include disposable gloves (double layers), particulate filter respirator mask with fluid shield protection, impermeable gowns and protective eye wear in the segregated area.

   5.4 Screw-capped buckets for centrifuges. Loading and unloading of samples should be carried out inside a Class II biosafety cabinet.

   5.5 Dedicated instruments for the testing of samples suspected for VHF.

   5.6 Eliminate the use of sharps and needles whenever possible, as sharps injuries are the main causes of laboratory-acquired VHF.

   5.7 Tests should be performed in a way which does not generate aerosols. Closed automated systems and instruments based on dry chemistry should be used.

5.8 Class II biosafety cabinet must be used when there is potential for creating aerosols. If this is not possible, such procedures should be avoided or be carried out in a BSL-3 containment laboratory.

5.9 Laboratory designed (including the furniture) for easy cleaning.

6.0 All work surface and equipment should be decontaminated appropriately.

6.1 Staff handling the tests should be trained and competent.

6. **Laboratory Tests**

Upon presentation of a possible case of VHF, the following tests relevant to the the immediate treatment and differential diagnosis may be performed e.g.:

- Blood films (following fixation, as described in Annex D) to look for malaria parasites on at least two occasions. Blood smears are not infectious after fixation in solvents.
- Two sets of blood cultures using routine blood culture bottles taken from separate vein punctures at least 30 minutes apart with a total volume per set of 20 to 30 ml.
- White blood cell and differential count and either haemoglobin or haematocrit.
- Urea and electrolytes
- Urine culture, if clinically indicated.

7. The laboratory should send specimens to the MOH-designated testing laboratory for Ebola and other VHF PCR testing by filling up all required information in request form (Annex B). Where in doubt, the laboratory should contact the National Public Health Laboratory for further assistance (see Annex C). There should be proper instruction and training in packaging before transport of sample between laboratories (see Annex A).

8. The laboratory should not attempt to perform any test for Ebola, including virus isolation on these specimens or send them to external parties without prior approval from MOH.

9. Should a sample be confirmed to be **Ebola positive**, **no virus isolation** should be done. Samples should only be processed in a **BSL-3 containment laboratory**. Should any laboratory wish to process an Ebola positive sample in a BSL-2 facility with BSL-3 practices, please contact MOH for assessment and approval.

Other measures

1. There should be an effective cleaning and disinfection procedures for laboratory equipment and accidental spills.

2. There should be proper disposal of all waste.

3. There should be a plan for the management of accidental staff exposure to potentially infectious material.

References

1. Interim Guidance for Managing Patients with Suspected Viral Hemorrhagic Fever in U.S. Hospitals – Update to previous recommendations (MMWR 1995; 44(25): 475-479). Centers for Disease Control and Prevention. 19 May 2005.

2. Interim Infection Control Recommendations for Care of Patients with Suspected or Confirmed Filovirus (Ebola, Marburg) Hemorrhagic Fever. BDP/EPR/WHO, Geneva March 2008. http://www.who.int/csr/bioriskreduction/interim_recommendations_filovirus.pdf

3. Management of Hazard Group 4 viral haemorrhagic fevers and similar human infectious diseases of high consequence. UK Department of Health. July 2012. http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1194947382005

4. Laboratory Precautions for Samples Collected from Patients with Suspected Viral Haemorrhagic Fevers. Commonwealth Department of Health and Aged Care, Canberra, Australia. 2001. http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-pubs-other-vhf.htm/$FILE/vhf_guide.pdf

5. Infection Prevention and Control Recommendations for Hospitalized Patients with Known or Suspected Ebola Hemorrhagic Fever in U.S. Hospitals. Centers for Disease Control and Prevention. 1 August 2014. http://www.cdc.gov/vhf/ebola/hcp/infection-prevention-and-control-recommendations.html

6. Management and Control of Viral Haemorrhagic Fevers and other highly contagious viral pathogens. European Network for Diagnostics of Imported Viral Diseases (ENIVD). 2001. http://www.enivd.de/netz.pdf

7. Interim Guidance for Specimen Collection, Transport, Testing, and Submission for Patients with Suspected Infection with Ebola Virus Disease. Centers for Disease Control and Prevention. 6 August 2014. http://www.cdc.gov/vhf/ebola/hcp/interim-guidance-specimen-collection-submission-patients-suspected-infection-ebola.html

ANNEX A

SPECIMEN PACKING AND TRANSPORT

For clinical samples, the following receptacles/material must be included in the packing:

1. Primary watertight and sealed inner receptacle

- Primary receptacle(s) must be water tight;
- Multiple primary receptacles must be wrapped individually to prevent breakage;
- Each receptacle must be labelled with sample ID;
- The entire contents of the primary receptacle are the diagnostic specimen(s).

2. Absorbent material

- Place absorbent material between the primary and secondary receptacles;
- Use enough material to absorb the entire contents of all primary receptacles;
- Acceptable absorbent materials include cellulose wadding, cotton balls, super-absorbent packets and paper towels.

3. Secondary watertight inner receptacle

- Use a watertight sealed plastic bag, plastic canister or screw-cap can.
- The secondary container should be externally disinfected - e.g. by wiping with hypochlorite (1,000ppm).

4. Sturdy outer packaging

- Use rigid outer packaging constructed of corrugated fibreboard, wood, metal or plastic, appropriately sized for the contents.
- Labels on outer packaging:
  - I. Sender's name and address
  - II. Recipient's name, contact number address
  - III. Biohazard label
  - IV. Name and telephone number of person responsible for shipment (normally sender should be the person-in-charge)

Note:

A. **ONLY** insert laboratory test instructions and description of materials in between of secondary receptacle and outer packaging.

B. **No dry-ice** should be placed in primary, secondary receptacles and air-tight containers.

C. Pins, staples and metal clips should **NOT** be used.

D. Direct transport, where van is preferred, should be arranged.

E. The sending laboratory must contact the receiving laboratory to confirm the specimen is on its way and to give the expected arrival time. The receiving laboratory should call the referring Laboratory to confirm safe receipt.

ANNEX B

Form # 5.4.a Test Request Form Ver 0.0

Clinical Diagnostic Services Laboratory
DMERI@DSO
27 Medical Drive #13-00
Tel: 64857258 / 64857242

Patient Information (PLEASE FILL UP COMPLETELY IN BLOCK LETTERS)	
Patient Name: NRIC No. / FIN No.: Date of Birth: Residential status: Resident ☐ Non-Resident ☐	
Requesting Doctor:	Requesting Medical Centre/ Hospital
Requesting Doctor Contact Number:	Diagnosis:
Specimen Collection Date:	Specimen Collection Time:
Type of Specimen:	

PCR Diagnostic Tests
1)
2)
3)

Special Instructions if any:

_____
Signature of Requesting Doctor

CDSL retains the right to reject any specimen which is less than unequivocally identified, or submitted without all of the testing information needed. Soiled or leaked specimen will not be tested.

ANNEX C

LABORATORY CONTACT DETAILS

DSO National Laboratories (designated testing laboratory)

1. Dr Jimmy Loh
   Contact No.: 6485 7242 / 9796 5585
   Email: jimmyloh@dso.org.sg

2. Mr Victor Koh
   Contact No.: 6485 7258 / 9819 2729
   Email: kweehong@dso.org.sg

National Public Health Laboratory

1. Dr Cui Lin
   Contact No.: 6357 7301 / 9388 7212
   Email: cui_lin@moh.gov.sg

2. Mr Roger Chua
   Contact No.: 6357 7302 / 9488 0312
   Email: roger_chua@moh.gov.sg

Annex D

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. This temperature is liable to coagulate IgG and invalidate serological tests. Based on experience with other viruses, laboratories may elect to use 57^{o}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^{o}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%, glutaradehyde 1% or greater, or 10% 10% buffered formalin for 15 minutes are satisfactory for inactivating the virus.

Adapted from the Laboratory Precautions for Samples Collected from Patients with Suspected Viral Haemorrhagic Fevers. Commonwealth Department of Health and Aged Care, Canberra, Australia. 2001.

Annex E

	European Network for Diagnostics of Imported Viral Diseases (ENIVD)	Advisory Committee on Dangerous Pathogens (ACDP) and Department of Health (DH), UK	Australia Public Health Laboratory Network	World Health Organization (WHO)	United States Centre for Disease Control and Prevention (CDC)
Risk Categories	None	1) Highly unlikely 2) Possibility of VHF infection 3) High Possibility of VHF infection 4) Confirmed	None	None	None
Designated Receiving Area (DRA)	Not described	Not described	• Must be physically separated from other areas by a door • Must be able to be sealed for decontamination • Must contain at least one Class 1, 2 or 3 biosafety cabinet, a lab sink, a hand washing sink, a refrigerator and a -20°C freezer	Not described	Not described
Containment	**Inactivated Specimens** • Class II biological safety cabinet following biosafety level 3 practices • Serum to be pre-treated with Triton XR-100. **Not inactivated Specimens** • Handled by experienced	**Possibility of VHF** • Sample testing carried out in closed system analysers at BSL-2 conditions. **High Possibility of VHF** • Testing at BSL-2 facility with some additional precautions • Samples should be inactivated. If not inactivated, specimens should be processed in a segregated area using a dedicated standalone	**Inactivated samples** • Processed as routine samples using standard (BSL2) laboratory precautions. **Non-inactivated samples** • BSL2 with closed system analyzers. Manual methods by experienced staff. • Proper disposal of waste fluid and decontamination	• Laboratory personnel handling potential HF clinical specimens should wear gown, gloves, particulate respirators and eye protection or face shields, or powered air purifying respirators (PAPR) when undertaking any other procedure that may generate aerosols.	• Process clinical specimens in a class II biological safety cabinet following biosafety level 3 practices AND with full face shield/goggles, masks to cover all of nose and mouth, gloves, fluid resistant / impermeable gowns. • Avoid aerosol-generating procedures (AGPs). **Virus isolation** • Biosafety level 4 facilities and procedures are required.

	personnel, in BSL3 facilities.	machine and sealed centrifuge machine. **<u>Confirmed VHF infection</u>** • All testing to be done at BSL-3 facility.	of instrument		