GUIDELINES FOR THE LABORATORY INVESTIGATION OF SUSPECTED EBOLA VIRUS DISEASE

07 August 2014

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1. INTRODUCTION

Specific diagnostic tests for the Ebola Virus Disease (EVD) and other haemorrhagic fevers are available from the Special Viral Pathogens Laboratory (SVPL), Centre for Emerging and Zoonotic Diseases of the National Institute for Communicable Diseases. The Laboratory offers a full repertoire of testing for laboratory investigation of EVD and other haemorrhagic fevers. In order to investigate these cases securely and safely the Laboratory operates the only Biosafety Level 4 laboratory in Africa.

This document summarizes the procedure for submitting, types and interpretation of testing for EVD. For further information related to the EVD outbreak and other related documents please refer to www.nicd.ac.za.

2. CASE DEFINITION

The case definition for suspected EVD cases is –

Person presenting with an acute onset of fever who has either:

- Visited or been resident in Guinea, Liberia, Sierra Leone or Nigeria in the 21 days prior to onset of illness

AND

- Had direct contact or cared for suspected/confirmed EVD cases in the 21 days prior to onset of illness, or been hospitalized in the affected areas

OR

- Has unexplained multisystem illness that is malaria negative

2.1 Differential diagnosis

Malaria is the most likely cause of an acute febrile in returning travellers from most African countries and has to be prioritized for testing as a likely cause of disease in such patients.

Other common causes of febrile illness in returning travellers from African countries include **Dengue fever, Hepatitis A, tick bite fever and typhoid. Lassa fever** is an important cause of haemorrhagic fever in the West African region in mainly rural areas where there is potential exposure to rodent urine.

Specialized testing for EVD is not warranted for patients without a compatible clinical picture and history or risk of possible exposure, even in the event of a history of travel to an affected Ebola area. The tests cannot be used to determine if the patient has been exposed to the virus and may develop the disease later. The tests are not indicated for healthy returning travellers.

3. PROCEDURE FOR SUBMISSION OF SPECIMENS FOR INVESTIGATIONS

STEP 1: REPORT THE SUSPECTED CASE TO THE NICD TO ALLOW A RISK ASSESSMENT TO BE CARRIED OUT AND GUIDE LABORATORY TESTING

- Contact the NICD Hotline ❫ **+2782-883-9920**

STEP 2: COMPLETE THE CASE INVESTIGATION FORM

- Fully complete the case investigation form (see appendix 1)

STEP 3: SUBMIT SPECIMENS FOR SPECIALIZED LABORATORY INVESTIGATION

- Submit both a clotted blood (red or yellow top tube) and EDTA treated tube (purple top tube) per patient
- The specimens should be packaged in accordance with the guidelines for the transport of dangerous biological goods (triple packaging using absorbent material) and transported directly and urgently to:

  Centre for Emerging and Zoonotic Diseases
  Special Viral Pathogens Laboratory
  National Institute for Communicable Diseases (NICD)
  National Health Laboratory Service (NHLS)
  No. 1 Modderfontein Rd
  Sandringham, 2131

- See section 4 for transport requirements
- Ensure the that completed case investigation form accompanies the specimens
- Samples should be kept cold during transport (cold packs are sufficient).

4. PACKAGING OF SPECIMENS FOR TRANSFER TO NICD

The principle of triple layer packaging should be followed (see below).

UN/WHO approved shipping containers for hazardous specimens are commercially available, e.g. SAF-T-PAK® (www.saftpak.com) or PATHOPAK®(www.intelsius.com) , or else safe packaging can be improvised (not for air transport) as indicated in the text box below (Figure 1a and b).

It is recommended that designated staff members per site are trained by approved provider in the packaging and transport of dangerous goods. The IATA of WHO websites may be consulted for international regulations and guidelines in this regard.

Primary specimen containers such as blood tubes (properly labeled) should be wrapped in sufficient absorbent material (paper towels or tissues) to absorb the entire contents in the event of leakage.

The wrapped primary containers must be placed in durable, leak-proof **secondary containers** such as several layers of sealed plastic bags or, preferably, rigid screw-cap metal, plastic or similar containers (suitable containers are usually available from hospital dispensaries). The secondary container should be taped closed to prevent leakage.

The secondary containers and data forms, sealed separately in plastic, must then be placed in a **rigid outer (tertiary) container s**uch as a fibre carton or polystyrene cold box with cold packs. Specimens, particularly whole blood, should not be frozen.

The outer wrapping should be addressed to:

The Centre for Emerging and Zoonotic Diseases, Special Viral Pathogens Laboratory, National Institute for Communicable Diseases, 1 Modderfontein Road, Sandringham, South Africa.

Contact telephone numbers: 011 386 6376 or 6339, 082 903 9131

The parcel should bear appropriate outer warning that it contains biohazardous material.

If transported by air, IATA regulations must be followed and appropriate labeling applied (refer to www.iata.org. In addition to completing an ordinary air waybill for parcels sent by air, it is necessary to complete a shipper's declaration for dangerous goods (refer to www.iata.org or your courier company).

Useful links:
The IATA of WHO websites may be consulted for international regulations and guidelines in this regard.

International Air Transport Association. Dangerous Goods Regulations. http://www.iata.org/publications/dgr/Pages/index.aspx, Accessed on 8 August 2014.

World Health Organization. Guidance on the regulations for the transportation of dangerous goods, 2013-2014. WHO/HSE/GCR/2012.12, Geneva, Switzerland. http://www.who.int/ihr/publications/who_hse_ihr_2012.12/en/, Accessed on 8 August 2014.

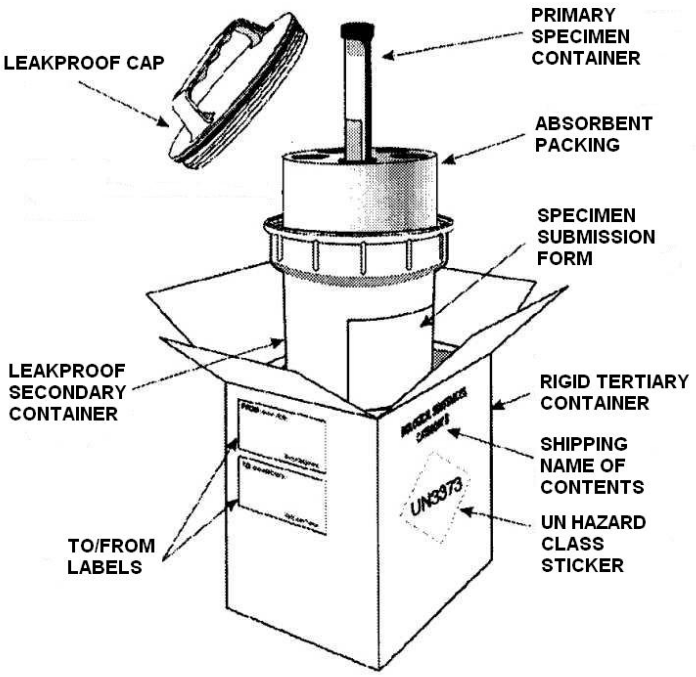

Figure 1a: Example of commercially available specimen packaging.

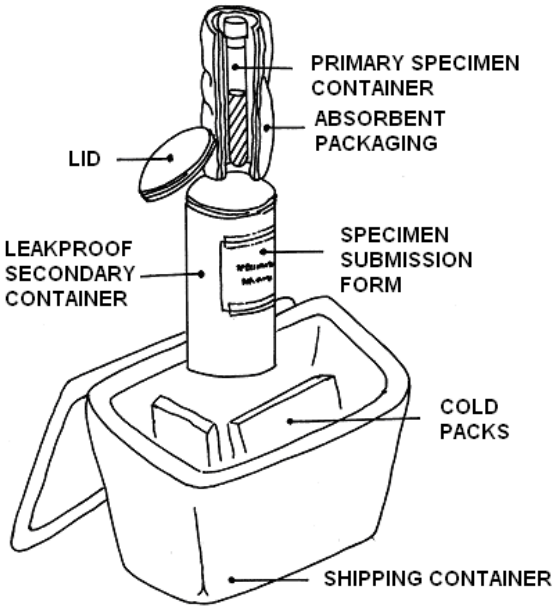

Figure 1b: Example of improvised specimen packaging.

4.1 Transport of specimens to NICD

Specimens are transported to NICD via:

- National Health Laboratory Service (NHLS) specimen transportation system for NHLS laboratories;
- As per internal arrangement for private pathology laboratories (e.g. Ampath, Lancet, Pathcare).
- The use of courier companies, as per internal arrangement, where possible is recommended to expedite the delivery of specimens

Specimens are delivered to the NICD Specimen Reception Office during office hours. **For after - hour** deliveries the specimens are deposited in a designated facility at the NICD Specimen Reception Office as directed by security staff at the main gate of the NICD Campus. It is recommended that the laboratory is forewarned of such deliveries by calling 011 386 6339 or 082 903 9131.

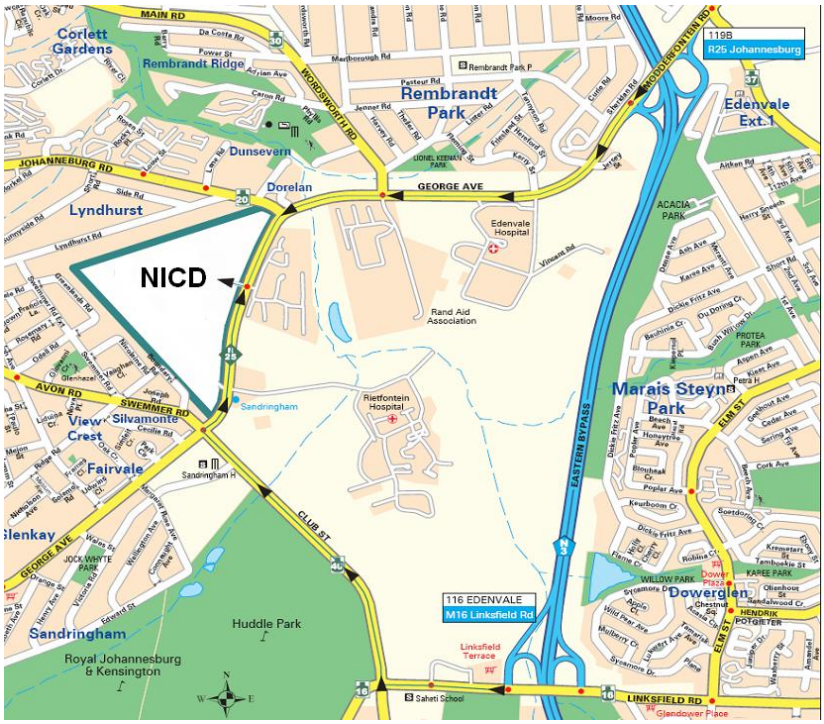

Figure 3: Road map to the NICD Campus in Sandringham

5. SPECIFIC EVD LABORATORY TESTS AVAILABLE AT THE NICD

The NICD offers a full repertoire of laboratory testing for EVD. Test requests need only specify for EVD or VHF investigation. The NICD will provide appropriate testing for each case.

Table 1: Summary of laboratory tests available at the NICD for EVD

Available tests	Turn-around time
Serology: fluorescent antibody test, IgG and IgM	24-48 hrs
Serology: ELISA, IgG and IgM	3-5 days
PCR	24-48 hrs
Virus isolation	21 days

NO SPECIMENS WILL BE PROCESSED WITHOUT A CASE INVESTIGATION FORM (APPENDIX 1 OR www.nicd.ac.za)

PLEASE NOTE THAT NON-SUBMISSION OF THE CASE INVESTIGATION FORM WILL CAUSE DELAYS IN PROCESSING OF SPECIMENS!

6. INTERPETATION OF SPECIFIC LABORATORY TESTS FOR EVD

In the acute phase of the disease, cases of EVD are diagnosed by identifying virus antigen or nucleic acid in the specimens, or by isolating (culturing) live virus. Detection of virus nucleic acid by reverse transcription-polymerase chain reaction (RT-PCR) takes 6-12 hours from the time of receiving the specimen in the laboratory, depending on whether or not there is need for nested (second round) tests. Isolating virus in culture can sometimes be achieved within 2 days but usually takes a week or longer.

In the convalescent phase of the disease, cases of EVD are diagnosed by identifying an antibody response. Preliminary IgG antibody tests can be completed within two hours of receipt of specimens and IgM tests within 3 hours, but overnight tests produce more reliable results.

It is extremely important to remember that even acute specimens for which virus antigen, RT-PCR and antibody tests are all negative, may nevertheless occasionally yield virus in culture some days later. Failure to appreciate this possibility has led to serious misunderstandings in the past and the premature removal of infection control measures.

Sometimes it is necessary to submit a further sample to clarify an ambiguous finding. For example, detection of IgG antibody on its own, without virus or IgM antibody, could indicate past infection not connected to the current illness, but sometimes IgG can appear in circulation slightly before IgM during convalescence.

It is almost equally important to eliminate a possible diagnosis of EVD as it is to confirm a diagnosis rapidly: failure to detect virus or viral nucleic acid in serum during the first 7 days of illness, or to demonstrate antibody two weeks after onset, constitutes a fair indication that one of the known African VHFs is not involved. However, viraemia may be of very short duration or absent. Hence, negative findings on samples taken early in the course of disease should be supported by antibody tests on further specimens taken in convalescence.

In emergencies results are made known telephonically or by fax as soon as possible, with written confirmation following later **(remember to include contact details (mobile telephone numbers are very important) for the person to whom results should be reported when submitting specimens)**.

APPENDIX 1

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division in the National Health Laboratory Service

Special Viral Pathogens Laboratory
Telephone (office hours): +27 11 386 6376
NICD Hotline (24-hour service): +27 82 883 9920

CASE INVESTIGATION FORM: REQUEST FOR EBOLA VIRUS DISEASE TESTING

PATIENT DETAILS

Surname:	Name/s:	
Date of birth:	Age:	Sex: Male Female
Contact telephone number/s:	Occupation:	
Physical home address:		

ATTENDING HEALTHCARE WORKER AND HEALTHCARE FACILITY DETAILS

Name of clinician:	Contact number/s of clinician:	
Healthcare facility name:	Location of healthcare facility:	
Hospital number:	Date of admission (dd/mm/yyyy):	Ward:

CLINICAL INFORMATION

A. Date of onset of illness (dd/mm/yyyy):

B. Clinical features (Tick appropriate box: yes, no, unknown)

Fever Yes □ No □ Unknown □
If yes, specify temperature___°C
Headache Yes □ No □ Unknown □
Muscle pain Yes □ No □ Unknown □
Joint pain Yes □ No □ Unknown □
Abdominal pain Yes □ No □ Unknown □
Sore throat Yes □ No □ Unknown □
Nausea Yes □ No □ Unknown □
Vomiting Yes □ No □ Unknown □
Diarrhoea Yes □ No □ Unknown □
Eschar Yes □ No □ Unknown □
Jaundice Yes □ No □ Unknown □
Bruising Yes □ No □ Unknown □
Bleeding Yes □ No □ Unknown □
Other, specify: _____

Rash Yes □ No □ Unknown □
If yes, specify
Distribution of rash:_____
Type of rash:
Macular Yes □ No □
Maculopapular Yes □ No □
Vesicular Yes □ No □
Petechial Yes □ No □
Vasculitic Yes □ No □

Bleeding Yes □ No □ Unknown □
If yes, specify
Epistaxis Yes □ No □
Haematuria Yes □ No □
Ecchymoses Yes □ No □
Haematemisis Yes □ No □
Melaena Yes □ No □
Other: specify:_____

C. Antimicrobial therapy

Has the patient received any antibiotics therapy during this illness? Yes □ No □ Unknown □
If yes complete the table below

Antibiotic	Route (po/IV /IM)	Date started	Date stopped	Duration (days) of treatment
		D D M M Y Y Y Y	D D M M Y Y Y Y	
		D D M M Y Y Y Y	D D M M Y Y Y Y	
		D D M M Y Y Y Y	D D M M Y Y Y Y	

Has the patient received any antimalarial therapy during this illness? Yes □ No □ Unknown □

If yes complete the table below

Antimalarial	Route (po/IV/ IM)	Date started	Date stopped	Duration (days) of treatment
		D D M M Y Y Y Y	D D M M Y Y Y Y	
		D D M M Y Y Y Y	D D M M Y Y Y Y	

D. Supportive management (Tick appropriate box: yes, no, unknown)

Patient requiring intensive care support Yes □ No □ Unknown □

Mechanical ventilation Yes □ No □ Unknown □

Dialysis Yes □ No □ Unknown □

Blood/blood product transfusion: Yes □ No □ Unknown □

Other: specify

_____-

LABORATORY INVESTIGATION RESULTS

FBC **RESULT** **DATE**

Haemoglobin: _____ _____/_____/_____

Platelet count: _____ _____/_____/_____

White cell count: _____ _____/_____/_____

Liver function tests

Total bilirubin: _____ _____/_____/_____

Direct bilirubin: _____ _____/_____/_____

AST: _____ _____/_____/_____

ALT: _____ _____/_____/_____

ALP: _____ _____/_____/_____

GGT: _____ _____/_____/_____

U & E:

Urea: _____ _____/_____/_____

Creatinine: _____ _____/_____/_____

RESULT **DATE**

Coagulation profile:

INR: _____ _____/_____/_____

PTT : _____ ____/_____/_____

D-dimers: _____ _____/_____/_____

Malaria tests:

Malaria smear: Pos □ Neg □

Malaria antigen: Pos □ Neg □

Blood culture: Date collected: _____/_____/_____

Status:

Other relevant tests and results (specify)

RISK FACTORS/ EXPOSURE HISTORY – during the 3 weeks prior to onset of symptoms
I WOULD ADD
if hospitalized or recievd medical care in these countries

Travelled to a country where EVD cases have occurred during the current outbreak Yes □ No □ Unknown □

(West African countries affected by outbreak and countries reporting imported cases)

History of contact with blood/body fluids of a patient with suspected/confirmed EVD Yes □ No □ Unknown □

History of contact with the immediate environment of a patient with suspected/confirmed EVD Yes □ No □ Unknown □

Handled or slaughtered bats or bush-meat animals in Guinea, Liberia or Sierra Leone Yes □ No □

Unknown □

Handled clinical/laboratory specimens from a patient with suspected/confirmed EVD Yes □ No □
Unknown □

Involved in the funeral preparations of a patient with suspected/confirmed EVD Yes □ No □
Unknown □

Had sex in the last 3 months with a patient with suspected/confirmed EVD Yes □ No □
Unknown □

PAST MEDICAL AND TRAVEL HISTORY

Underlying illness : Yes □ No □ Unknown □
If yes, give details:

Travel outside of South Africa in the four weeks prior to onset of illness? Yes □ No □ Unknown □
If yes, details:

Country visited	Location/s visited within country:	Date of arrival (dd/mm/yyyy):	Date of departure (dd/mm/yyyy):

Reason for travel (e.g. business, tourist, visiting friends/family), specify:
_____

Activities (e.g. hiking, walking, hunting), specify: _____

Yellow fever vaccine received: Yes □ No □ Unknown □

Antimalarial chemoprophylaxis received: Yes □ No □ Unknown □

DIFFERENTIAL DIAGNOSES

List current differential diagnoses considered:
_____
_____

Appendix 2

USEFUL CONTACT NUMBERS

REQUIREMENT	CONTACT NUMBER	CONTACT PERSON/S
Reporting of suspected case	082 883 9920	Pathologist on call
Clinical advice regarding suspected cases	082 883 9920	Pathologist on call
Queries regarding laboratory testing	011 386 6376 011 386 6338	Dr Jacqueline Weyer
Queries regarding laboratory results	011 386 6376 011 386 6338	Dr Jacqueline Weyer