Standard Operating Procedure
for collection, packaging and transportation of blood samples for Ebola Virus Disease diagnosis

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1.0 Scope

This standard operating procedure describes the procedure for collection, packaging and transportation of blood samples from suspected Ebola patients presenting at any of the Ebola Treatment Centres (ETCs) and/or health centres/hospitals within The Gambia.

2.0 Purpose

The purpose of this standard operating procedure is to ensure safe and timely blood sample collection, packaging and transportation to the regional reference laboratory (Pasture Institute, Dakar, Senegal).

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Health Facility Code: Copy No: Reason for change: Not applicable.
3.0 Safety and Precautions

Ebola virus is a highly contagious pathogen (category IV) and it must be handled as such. Therefore, safety precautions appropriate for category IV must be followed when collecting blood samples from suspected Ebola patients. In order to prevent direct contact with blood or other secretions from patients, appropriate personal protective equipment must be worn during sample collection and packaging. Hands must be washed with 0.05% of active chlorine before and after each stage of the donning off of the PPE. Disinfect all surfaces and equipment with 0.5% active chlorine. All sharp items such as lancets, and all other materials that have come in contact with the patient should be disposed off immediately after use in an appropriate (i.e., puncture resistant) biohazard container.

4.0 Definitions and abbreviations

4.0.1 Definitions

1. **Ebola Virus Disease**: It is a highly contagious and fatal disease caused by the Ebola virus. Transmission is mainly via direct contact with fluids or secretions from an infected person (dead or alive).

2. **Category IV**: This refers to pathogens that can cause severe to fatal diseases in humans or animals when exposure occurs. They require a high containment level (BSL 4) during analysis or handling in a laboratory setting.
3. **Health Facility Code**: This is the unique code assigned to each facility within the country. This code shall be used to identify individual laboratories within these health facilities.

4. **Copy Number**: This is the unique number of the copy of the SOP that is assigned to each particular laboratory. Each laboratory has its unique copy number.

5. **Number of copies**: This is the amount of SOP documents that have been printed and disseminated to the various laboratories across the country. The number of copies shall be increased when the need arises.

6. **Buddy**: This is the other laboratory personnel who will be involved in assisting the one collecting the sample.

4.0.2 Abbreviations

- BSL 4 – Biosafety Level 4
- EDC – Epidemiology and Disease Control
- EDTA – Ethylene diamine tetra-acetic acid
5.0 Personnel responsibilities

The head of the laboratory must ensure that all laboratory personnel under his/her purview who are required to collect samples from suspected Ebola patients are knowledgeable of the procedure for safe sample collection, packaging and transportation. New employees are to be trained and certified for competence by trained personnel before they can collect and handle patient samples. It is the responsibility of the laboratory personnel assigned to collect, package and transport blood samples from suspected Ebola patients to strictly adhere to this SOP.

6.0 Specimen

6.0.1 Specimen type
Whole blood

6.0.2 Specimen collection

Specimens will be collected by venipuncture.

7.0 Materials and supplies

- Blood collection tube (preferably EDTA Vacutainer tubes)
- Needles and syringes/Vacuum extraction needles
- Tourniquet (single-use)
- Skin antiseptic solution (0.05% active chlorine)
- Dry cotton wool
- Puncture-resistant sharps container

1. Gauze pads
2. Alcohol pads
3. Adhesive bandage
4. Tray for assembling blood collection kits
5. Rack for holding blood sample tubes
6. Permanent marker for labeling
7. 0.5% active chlorine
8. Biohazard bags (leak-proof)
9. Waste bin
10. Liquid soap (preferably Lab Guard)
11. UN2814 specified sample packaging box with triple packaging materials
12. **Personal Protective Equipment (PPE):**
    1. Latex examination gloves
    2. Rubber boots/ disposable shoe covers
    3. Protective clothing (scrubs)
    4. Coverall
    5. Plastic Apron/Heavy duty apron
    6. Mask (N95)
    7. Goggles/Face shields

**8.0 PROCEDURE**

**8.0.1 PPE DONNING**

1. Before entering patient room/ward, assemble all the PPE, sample collection and packaging materials.

2. Wear first layer of protective clothing (scrubs) after removing your outermost clothing and perform hand hygiene.
Note: Donning and donning off of PPE must be done in a specified area different from the patients’ ward/area.

1. Put on rubber boots one at a time (Note: always put on a boot size bigger than your normal shoe size for easy removal).
4. Put on the coverall by opening the zip and inserting both legs into the pairs of trousers of the coverall (one leg at a time), make a thumb hole and zip it up to the neck and hood up.

1. Put on the plastic apron/heavy duty apron and tie the two projections of the apron behind your back in a rift-knot-like form.

   Note: Use heavy duty apron, gloves and coverall if the patient is bleeding profusely.
2. Put on the N95 mask in a manner that fits well on your face.

3. Put on the goggles

4. Put on first pair of gloves (inner gloves) and ensure cuffs are tucked under the sleeves of the coverall.
5. Put on the second pair of gloves and ensure cuffs are tucked over sleeves of the coverall

6. Seal the outer gloves with adhesive tape if it is raining.

Note: Ask your buddy to check and confirm whether you are properly donned or not. Besides, you must check yourself on a wall mirror to further confirm that you are properly donned.

1. SAMPLE COLLECTION

NOTE: Two laboratory personnel must be involved in the sample collection and packaging process. One should be responsible for sample collection whilst the other personnel proceed to the designated area for packaging.
1. After putting on the appropriate PPE, label the sample collection tube with the patient’s details including the date of collection (labeling should be done before entering the ward/patient area).

2. Walk into the patient room with your blood sample collection kits.

3. Inform patient of the purpose and method for collecting the blood sample.

4. Tie a disposable tourniquet around the patient’s arm to visualize the veins.

5. Disinfect the selected puncture site with an alcohol pad and let it air-dry (approximately 15-20 seconds).

6. If using a vacuum extraction system, fasten the needle in the Vacutainer sleeve and put it in place (in kits’ tray).

7. Alternatively, when using a needle and syringe, fasten the needle into the hub of the syringe and put it on kits’ tray.

8. Position the needle at a 45 degrees angle to the arm and insert into the selected vein with the beveled-edge facing upwards.

9. Collect 4-5ml of blood
10. When using a Vacuum extraction method, push the Vacutainer tube into the needle to collect the blood.

11. When using a needle and syringe, loosen the tourniquet after collecting the blood and place a small pad of dry cotton wool on the punctured site as you withdraw the needle out of the vein. Ask the patient to bend the arm or help bend the arm with the cotton wool in place to stop the bleeding.

12. Dispense the blood by piercing the needle right through the top part of the rubber stopper of the Vacutainer.

13. When all the blood is collected into the Vacutainer, pull off the needle and syringe and appropriately dispose off into the sharps container.

14. If a vacuum extraction system is used, unplug the needle and dispose into the sharps container.

**DO NOT RECAP THE NEEDLE!**

15. Make sure that the punctured site is dry, if not place a small bandage on the punctured site to stop the bleeding. Remember to discard the cotton wool applied on the punctured site into a biohazard bag.

16. Bath the tube containing the blood sample in 0.5% active chlorine solution and place on a rack.
17. Hand over the specimen to the other laboratory personnel (buddy) for packaging.

18. Proceed to the designated donning off area and remove PPE in the following sequence as described below:
Note: Your buddy has to help you with the disinfection process using a spray bottle containing 0.5% active chlorine solution.

**DONNING OFF PPE**

1. Spray the front and back of the suit with 0.5% active chlorine solution disinfectant

2. Disinfect the outer pair of gloves by spraying with 0.5% active chlorine solution.
3. Disinfect the boots by asking your buddy/partner to pour 0.5% active chlorine solution on both boots into an empty container.

4. Disinfect the apron by spraying it with 0.5% active chlorine solution.

5. Remove the apron by untying from the back and bend a little bit forward. Then remove the apron over the head from the back.

1. Turn contaminated outside toward the inside.

2. Fold or roll into a bundle and discard into the disposable biohazard bag or reusable waste container if the apron is a reusable type (heavy duty).
3. Remove the coverall by unzipping it and remove it in an inside out manner.
National Public Health Laboratories (MoH & SW)
Serology Laboratory
Kotu Layout
Person responsible: Head of Laboratory

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4. Remove the goggles by pulling it away from your face and move it upwards to completely remove it.

5. Remove the outer pair of gloves by grasping the outside edge near the wrist and then peel away from hand, turning the glove inside-out. Then hold in opposite gloved hand (i.e the other hand).
6. Disinfect the inner pair of gloves by spraying with the 0.5% active chlorine.
7. Remove the mask by pulling it away from your face and move it upward to completely remove it.
8. Remove the rubber boots by using one foot to step on the backside of the other.

9. Disinfect the gloved hands by spraying with the 0.5% active chlorine.
10. Remove the inner pair of gloves

11. Soak hands in 0.05% active chlorine and soap and clean water.
**Note:** All reusable PPE are to be soaked into a container containing 0.5% active chlorine solution during the donning off process.

### 9.0 Specimen packaging

1. Ensure that all tubes are tightly closed to avoid any leakage of the sample.

2. Wrap every tube in an absorbent material like dry cotton wool and place it in a watertight, leak-proof screw-cap container.

3. Wrap the sample tube (EDTA) with dry cotton wool or other suitable absorbent material and place it in the water-tight, leak-proof container (secondary container) provided.

4. Collect four frozen ice packs from the freezer (-20 degrees Celsius or colder) and place them on the sides of the UN specified cold box (UN 2814) ensuring that the secondary container is in the middle of the ice packs.

5. Place the case investigation forms in a leak-proof, zip-lock plastic bag to prevent them from becoming contaminated or destroyed by the wet ice packs and put them inside the cold box on top of the secondary packaging.
6. Seal the cold container and disinfect the outer packaging with 0.5% active chlorine solution before transportation.

10.0 Transportation and receiving of samples
Samples should be transported at 4-10 degrees Celsius within 24 hours after collection.

Transport all samples from the point of collection to the regional reference laboratory for Ebola (Pasteur Institute, Dakar, Senegal) in consultation with relevant authorities (NPHL).

Clearance must be obtained from the Ministry of Health & Social Welfare prior to departure.

The EVD regional reference lab in Dakar should be notified prior to transportation of samples.

Samples will be transported in the designated vehicle to Dakar, Senegal.
11.0 References

1. WHO blood sample collection protocol, 2014- “How to safely collect blood samples from persons suspected to be infected with highly infectious blood-borne pathogens (e.g. Ebola).

2. Kenyatta National Hospital, August 2014 – “Standard Operating Procedure on sample collection, packaging and transportation-KEMRI/CDC.”


5. WHO, 2010, Generic guidance for development of SOPs, P14-39
APPENDICES

APPENDIX 1:

List of Contributors

<table>
<thead>
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APPENDIX 2:

List of certified personnel trained on this SOP

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<tr>
<th>Personnel</th>
<th>Designation</th>
<th>Signature</th>
<th>Date of Training</th>
<th>Supervisor</th>
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ANNEX 3:
MINISTRY OF HEALTH AND SOCIAL WELFARE

Ebola case investigation and recording sheet

Date of case detection ___/___/____

Case reported by (tick the box and specify):

☐ Mobile team, n° ____________
☐ Hospital ________________

Form filled in by (last and first name)

Information passed on by (last and first name)

Relationship with the patient

Case ID number: ______________________

Health centre ______________________

Other: _______________________________________

Information passed on by (last and first name)

Relationship with the patient

Nickname: ______________________

Patient identity

Surname __________________ Second Names __________________ First Names __________________

Son/daughter of (name of father/mother) ______________________________________________

Date of birth ___/___/____ age (years) ______ Sex ☐ M ☐ F

Ordinary residence: Head of household (last and first name) ____________________________

Village/neighborhood of residence ____________________ District _________ Region __________

GPS coordinates of domicile: Latitude _____________________ Longitude _____________________ Nationality: __________________________

Ethnic group: ____________________________

Patient’s profession (tick the appropriate box and provide details if necessary)

☐ Planter ☐ Homemaker ☐ Child ☐ Hunter/Bush meatretailer
□ Health-care worker, specify: health-care facility ____________________ Qualification ____________
□ Pupil/Student □ Other (specify) ______________________

Patient’s condition
Condition of the patient when found □ Alive □ Dead
If deceased, date of death ___/___/___
Place of death: □ Community, village/neighborhood ____________________ District __________
□ Hospital, name and department ____________________ District __________ Burial place, name
of village/neighborhood __________________________ District __________

History of present illness
Date on onset of symptoms ___/___/___
Name of the village where the patient became ill ____________________ District __________
Has the patient moved around since he/she became ill? □ Yes □ No □ Don’t Know
If the answer is “yes”, complete the list indicating villages, health-care facilities, and districts:
Village ____________________ Health-care facility ____________________ District __________
Village ____________________ Health-care facility ____________________ District __________
Village ____________________ Health-care facility ____________________ District __________

Clinical
Does the patient show any of the following symptoms *(tick all applicable)*

Has the patient had a fever?  
[ ] Yes  [ ] No  [ ] Don’t Know

If so, date of fever onset:  ___/___/___

Does the patient have or had any of the following symptoms *(tick the corresponding boxes and provide details if necessary)*:

1. headaches  [ ] Yes  [ ] No  [ ] Don’t Know
2. diarrhoea  [ ] Yes  [ ] No  [ ] DON’T KNOW
3. stomach pain  [ ] Yes  [ ] No  [ ] DON’T KNOW
4. vomiting  [ ] Yes  [ ] No  [ ] DON’T KNOW
5. lethargy  [ ] Yes  [ ] No  [ ] DON’T KNOW
6. anorexia  [ ] Yes  [ ] No  [ ] DON’T KNOW
7. muscular pain  [ ] Yes  [ ] No  [ ] DON’T KNOW
8. difficulty swallowing  [ ] Yes  [ ] No  [ ] DON’T KNOW
9. difficulty breathing  [ ] Yes  [ ] No  [ ] DON’T KNOW
10. intense coughing  [ ] Yes  [ ] No  [ ] DON’T KNOW
11. skin rash  [ ] Yes  [ ] No  [ ] DON’T KNOW
12. bleeding at injection points  [ ] Yes  [ ] No  [ ] DON’T KNOW
13. bleeding gums (Gingivitis)  [ ] Yes  [ ] No  [ ] DON’T KNOW
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Person responsible: Head of Laboratory

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☐ bleeding in eye (conjunctival injection) ☐ Yes ☐ No ☐ DON'T KNOW
☐ dark or bloody stool (melaena) ☐ Yes ☐ No ☐ DON'T KNOW
☐ vomiting of blood (hematemesis) ☐ Yes ☐ No ☐ DON'T KNOW
☐ nose bleed (epistaxis) ☐ Yes ☐ No ☐ DON'T KNOW
☐ vaginal bleeding outside of menstruation ☐ Yes ☐ No ☐ DON'T KNOW

Exposure risk

2. Has the patient been in contact with a suspected or confirmed case in the 3 weeks preceding the onset of the symptoms? ☐ Yes ☐ No ☐ Don’t Know
If so, specify: Last name ___________________________ First name ___________________________
At the time of contact, was the suspected case ☐ alive or ☐ dead? If dead, date of death ___/___/___ Date of last contact with the case ___/___/___

3. Was the patient hospitalized or has he/she visited a hospital nearby in the 3 weeks preceding the onset of the symptoms? ☐ Yes ☐ No ☐ Don’t Know
If so, where ___________________________ when (dates) ___/___/___ - ___/___/___

4. Has the patient seen a traditional healer in the 3 weeks preceding the onset of the symptoms?
   ☐ Yes ☐ No ☐ Don’t Know
If so, give name: ___________________________ Village ___________________________ District ___________________________
Where and when did the consultation take place? Place ___________________________ Date: ___/___/___
Has the patient received traditional treatment?  □ Yes  □ No  □ Don’t Know
If so, specify the type of traditional treatment: ___________________________________

5. Has the patient attended any funerals in the 3 weeks preceding the onset of the symptoms?
   □ Yes  □ No  □ Don’t Know
If so, last and first name of the deceased: __________________________________________

6. Has the patient had contact with any wild animals in the 3 weeks preceding the onset of the symptoms?
   □ Yes  □ No  □ Don’t Know
If so, kind of animal ___________________________ Locality __________________ Date ___/___/___

7. Has the patient worked or spent time in a mine/cave inhabited by bat colonies in the 3 weeks preceding the onset of the symptoms?
   □ Yes  □ No  □ Don’t Know
If so, name of the mine_____________________ Locality __________________ Date ___/___/___

8. Has the patient travelled in the 3 weeks preceding the onset of the symptoms?
   □ Yes  □ No  □ Don’t Know
If so, where to __________________________ and when ___/___/___ to ___/___/___

Specimen collection
Question for the investigation team: after having provided clear and full information to the patient (or in absentia to his/her family or legal guardian) did you obtain his/her express and/or informed consent to the collection of specimens?
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Name of specimen? □ Blood □ Urine □ Saliva □ Biopsy □ Stool

□ Yes □ No □ Don’t Know

□ Did you collect specimens? □ Yes □ No □ Don’t Know

If so, when ___/___/___

Transfer of the patient to hospital
To be completed ONLY by mobile teams and health centres

Was the patient taken to hospital? □ Yes □ No

If so, name of hospital __________________________ Date of transport ___/___/___

Updated information provided from the isolation unit
To be completed ONLY by the hospital OR the surveillance office

Was the patient referred to an isolation area? □ Yes □ No

If so, name of hospital __________________________ Date of hospitalization ___/___/___

Family member(s) accompanying the patient, last and first name __________________________

Date of discharge ___/___/___ OR Date of death ___/___/___
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Laboratory data

The specimen tested was collected from: ☐ Sick person  ☐ Recovering patient  ☐ Post-mortem

Date taken  ____/____/____  Date result received  ____/____/____  Lab ID ______________________

Type of specimen  ☐ Blood sample using dry tube  ☐ Blood using anticoagulants

☐ Saliva  ☐ Stool / Urine

☐ Biopsy  ☐ Other, specify ________________

Results

Antigen detected  ☐ pos  ☐ neg  ☐ NA  Date  ____/____/____

IgM serology  ☐ pos  ☐ neg  ☐ NA  Date  ____/____/____

IgG serology  ☐ pos  ☐ neg  ☐ NA  Date  ____/____/____

RT-PCR  ☐ pos  ☐ neg  ☐ NA  Date  ____/____/____

Virus culture  ☐ pos  ☐ neg  ☐ NA  Date  ____/____/____

Immunohistochemical staining  ☐ pos  ☐ neg  ☐ NA  Date  ____/____/____

Immunofluorescence  ☐ pos  ☐ neg  ☐ NA  Date  ____/____/____

---

Outcome (to be verified 4 weeks after onset of symptoms)
alive  dead
in case of death, date __/__/____

Final case classification (tick the appropriate box)

☐ Suspected  ☐ Probable  ☐ Confirmed  ☐ Non-case

Laboratory-related annexes

Guidelines for the collection of clinical specimens during field investigation of outbreaks
1. Put blood in EDTA not in glass containers
2. Do not submit specimen preserved in Heparin tubes
3. Specimen should be stored at temperatures not exceeding 4° Celsius
4. Specimen other than blood may be submitted upon consultation with CDC
5. Standard labelling should be applied for each specimen
6. Name of person who collected the sample should be written
7. Test(s) requested
8. Date of sample collection
9. Lab number
10. Type of specimen being shipped

NON-CASE:
Any suspected or probable case with a negative laboratory result. Non-cases are those which showed no specific antibodies, RNA, or specific detectable antigens.
Contact tracing: Standard definition of Ebola contacts

**Ebola case contacts:**
Any person having had contact with Ebola virus disease in the 21 days preceding the onset of symptoms in at least one of the following ways:

1. has slept in the same household with a case
2. has had direct physical contact with the case (alive or dead) during the illness
3. has had direct physical contact with the (dead) case at the funeral
4. has touched his/her blood or body fluids during the illness
5. has touched his/her clothes or linens
6. has been breastfed by the patient (baby)

**Contacts of dead or sick animals:**
Any person having had contact with a sick or dead animal in the 21 days preceding the onset of symptoms in at least one of the following ways:

7. has had direct physical contact with the animal
8. has had direct contact with the animal’s blood or body fluids
9. has carved up the animal
10. has eaten raw bush-meat

**Laboratory contacts:**
Any person having worked in a laboratory in the 21 days preceding the onset of symptoms in at least one of the following ways:

11. has had direct contact with specimens collected from suspected Ebola patients
12. has had direct contact with specimens collected from suspected Ebola animal cases
Important note: During an epidemic, these definitions may be changed to correspond to the local event.

Other infection risk factors include: contact with a hospital where Ebola cases are being treated; infection; or vaccination in the 21 days preceding the onset of symptoms.