Guiding notes for collection, storage and shipments of Ebola specimens

General instructions

1. Diagnostic specimens should be handled with extreme caution and sent to an appropriate laboratory "BSL 4 facility".
2. Inform the national reference laboratory before sending the shipment of specimens so that the necessary arrangements can be made for receiving it. This laboratory may need to forward the specimens to a WHO Collaboration Centre (WHO CC) laboratory. In this case **the WHO CC laboratory should be contacted as soon as possible.**
3. Wear PPE correctly to avoid any risk of contamination when taking specimens. Collect the required specimens in accordance with the sample collection procedures described in the guidelines.[1]
4. Store and package the specimens as described in the safety instructions.[2]
5. Contact the national reference laboratory again when the specimens are ready for shipping to make sure it is ready to receive them. Double-check with the laboratory that the address and shipping route are correct.
6. Send the specimens to the national reference laboratory. The specimens must be packaged using triple packaging requirements.[2] Relevant clinical and epidemiological information **must** be attached to the laboratory request form enclosed with the specimens.
7. 24 hours after shipment, verify the arrival of the specimens at the national reference laboratory

Type of sample and timing:

1. Acute phase whole blood obtained from a patient within 7 days of onset of illness.
2. Convalescent sera collected from patients at least 14 days after onset of illness. Paired serum samples are ideal, usually collected 7-20 days apart.

Basic safety precautions

1. As Ebola virus samples is considered as one of category A samples and should be handled in BSL4 facilities, samples collection should be handled with the most bio-risk measures regarding place and procedure.
2. Do not try to separate acute phase sera from blood clots (a procedure which may significantly increase the risk of accidental infection). The use of sealed sterile dry Vacutainer tubes is recommended. Ideally, blood samples should be kept in their original tube and stored at 4 C to allow PCR / virus isolation. To separate serum "and avoid hemolysis which can affect PCR results" just keep the tube stand upright "no movement or shaking" till RBCs are clotted down and serum is separated above
3. Use latex or nitrile gloves when taking and handling specimens. Dispose of gloves between patients and replace with a fresh pair. Do not attempt to clean and reuse gloves as this may promote the spread of pathogens from patient to patient. In addition, unnoticed damage to gloves is common, and places the healthcare worker at risk.
4. Wear protective clothing (gown, coat or apron, masks and googles) when collecting samples.
5. Discard used needles directly into sharps box, without recapping them.
6. Work areas and surfaces should be organized and disinfected with 1% household bleach daily or with a change in collection team. Use 10% bleach to clean up spills after wiping the surface

clean. Personnel carrying out cleaning or decontamination should wear a protective coat and thick rubber gloves.

7. Contaminated non-disposable equipment or materials should be soaked in 1% household bleach for 5 minutes. Before use wash in soapy water and sterilize if necessary.
8. Heavily soiled disposable items should be soaked in 10% household bleach before incineration or disposal.

Labelling and identification of specimens

Each patient should be assigned a unique identification number by the collection team. This unique identification number and the patient name should be present on all specimens, epidemiological data forms, and the laboratory request and used as a common reference

Laboratory form

1. A case investigation form should be completed for each patient at the time of collection. A laboratory request must also be completed for each specimen. The laboratory may require other information to select and interpret the necessary tests; this may include:
    - Patient information: age (or date of birth), sex, complete address
    - Clinical information – date of onset of symptoms, clinical history, risk factors or contact history where relevant, antimicrobial drugs taken prior to specimen collection
    - Laboratory information – acute or convalescent specimen, other specimens from the same patient.
2. The receiving laboratory should record the date and time when the specimen was received, name and initials of the person receiving specimen, and a record of specimen quality.

Storage of specimens

1. Specimens taken for viral isolation are acceptable for culture after two days if maintained in type specific media at 4-8°C. For longer periods, freeze these specimens as directed by expert advice, as infectivity may be altered. For prolonged storage periods, preservation at –70°C may be indicated
2. Specimens for antigen or antibody detection may be stored at 4-8°C for 24-48 hours, or at –20°C for longer periods. Some specimens may require special handling, for example freezing, so specific instructions should always be sought prior to collection. Sera for antibody detection may be stored at 4-8°C for up to 10 days. It is important to avoid unnecessary freeze-thaw cycles

Packaging and labelling of specimens

Detailed packaging, documentation, and handling requirements for the international transport of infectious materials are contained in the regulations of the International Air Transport Association (IATA) and in documentation of the International Health Regulations

Transport of specimens

Before transport, the collection team should notify the receiving laboratory of all shipping and specimen details in advance of specimen arrival. In many cases, initial surface transportation of

specimens from the field site to transport facilities may be required prior to shipment to processing laboratories. If international transport is necessary, authorization to import the specimens should be organized by the laboratory, which should also inform the sender of receipt or non-receipt of the specimens

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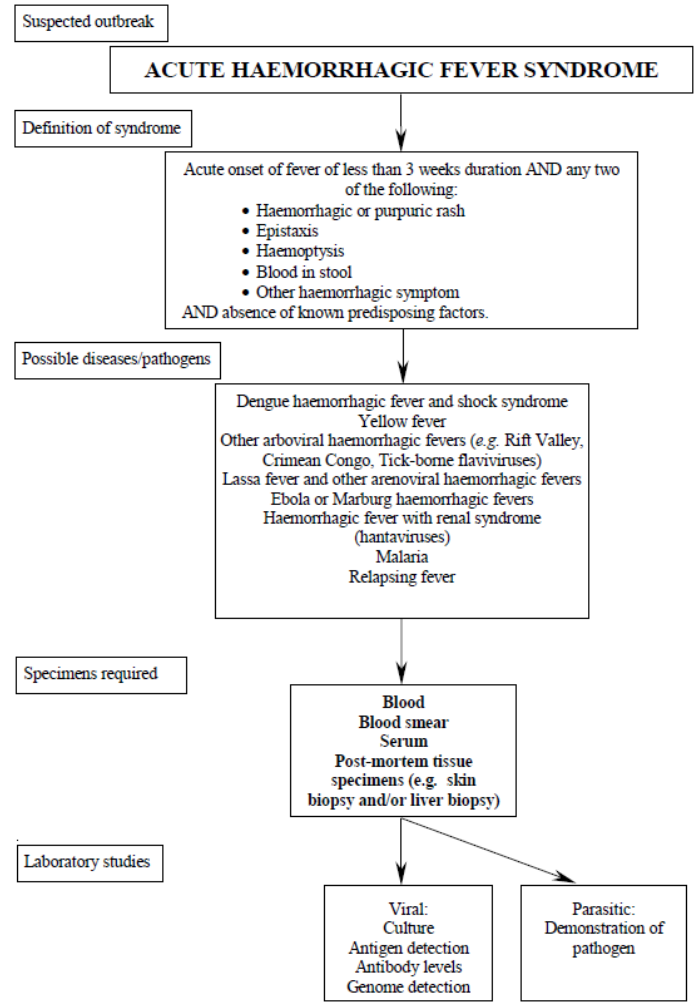

Laboratory tests used in diagnosis include:[3]

Timeline of Infection	Diagnostic tests available
Within a few days after symptoms begin	• Antigen-capture enzyme-linked immunosorbent assay (ELISA) testing • IgM ELISA • RT Polymerase chain reaction (RT- PCR) • Virus isolation
Later in disease course or after recovery	• IgM and IgG antibodies
Retrospectively in deceased patients	• Immunohistochemistry testing • PCR • Virus isolation

References:

1. Guidelines for the collection of clinical specimens during field investigation of outbreaks (WHO/CDS/CSR/EDC/2000/4)
http://www.who.int/csr/resources/publications/surveillance/WHO_CDS_CSR_EDC_2000_4/en/index.html

2. Guidance on regulations for the Transport of Infectious Substances 2011-2012
http://www.who.int/ihr/publications/who_hse_ihr_20100801_en.pdf

3. Ebola Hemorrhagic Fever diagnosis
http://www.cdc.gov/vhf/ebola/diagnosis/index.html