

Laboratory diagnosis of Ebola virus disease

19 September 2014

Important notes for laboratory staff

- Ensure that sufficient stocks of appropriate Personal Protective Equipment (PPE) and UN specimen triple packaging systems are available;
- Staff should be appropriately trained in putting on (donning) and removing PPE;
- Staff involved in specimen collection should be trained in how to collect, store, package and ship specimens, following national/international guidelines.

1. Specimen collection

The incubation period (the time from infection with the virus to the onset of symptoms) for EVD is 2-21 days. Patients are infectious when they start developing symptoms, namely fever (temperature > 38.5°C), diarrhoea, and haemorrhagic signs (bleeding). The bodies of deceased people are also infectious.

All Ebola cases (probable and suspected) should be referred to a designated Ebola Treatment Centre (ETC) or appropriate health care facility where trained medical staff should safely collect the appropriate specimens.

The timing of specimen collection

- Specimens for molecular detection should ideally be taken when a patient exhibits symptoms that meet the case definitionⁱ of EVD.
- If specimens are collected less than 3 days after onset of symptoms, additional specimens will be needed if the test result on the first specimen is negative. The second specimen should be collected at least 48 hours after the first specimen.
- Whole blood for serological testing can be collected after 8 days of onset of symptoms with strict infection prevention and control measures adhered to throughout the process, including waste disposal and disinfection. Refer to the WHO laboratory biosafety manual 3rd editionⁱⁱ for appropriate biosafety practices.

It is recommended that the following specimens be collected for the diagnosis of EVD:

- Whole blood in EDTA (a minimum volume of 4mL), collected in plastic tubes from live patients;
- Oral swabs stored in a universal transport medium, collected from deceased patients* or in situations where blood collection is not possible e.g. children. Swab collection from live patients is not recommended due to lower sensitivity for reverse transcription polymerase chain reaction (RT PCR) and antigen detection.

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Samples can be stored at room temperature for up to 24 hours. If samples can only be transported and tested within one week, store them between 0-5°C for further testing by RT-PCR. Store at -70 °C for long-term storage, and ship on dry ice if the specimen is referred to a WHO Collaborating Centre. Avoid freeze-thaw cycles.

* Trained personnel (laboratory technicians/epidemiologists/medical staff) should collect oral swabs from deceased patients in the community.

2. Laboratory biosafety recommendations

It is the responsibility of the institute/laboratory to perform a risk assessment and decide on appropriate biological risk mitigation controls. Any testing for the presence of Ebola virus, its ribonucleic acid (RNA) or antibodies against Ebola should be performed in appropriately equipped laboratories by staff trained in the relevant technical and safety procedures. National guidelines on laboratory biosafety should be followed under all circumstances.

It is recommended that countries without appropriate biosafety capacity to perform a laboratory diagnosis on cases under investigation (suspected and probable) should send specimens to a designated WHO Collaborating Centre for Viral Haemorrhagic Fevers (WHO CC for VHF) (Step 4).

Filoviruses are highly infectious agents and strict precautions must be applied when handling specimens for diagnosis. Laboratory tests on the non-inactivated virus present an extreme biological risk^{iii,iv}. Proper precautions and engineering control (i.e. facility and equipment) must be observed at all times, in accordance with the issues identified in the risk assessment for each procedure.

Biosafety recommendations for laboratories conducting diagnostic testing for EVD with appropriate biosafety BSL3/BSL4 facilities

- Virus isolation should be done only in a maximum containment BSL4 laboratory. Ensure safe and secure handling and storage of the virus isolates and other specimens from accidental or deliberate release.
- The inactivation of specimens, depending on the detection protocol used, should be performed under BSL3 conditions.
- For non-inactivated samples, RT PCR and enzyme-linked immunosorbent assay (ELISA) testing can be performed at a BSL3 laboratory.
- If samples have been inactivated (i.e. cell lysis) RT PCR and ELISA testing can be performed at a BSL2 laboratory.

Biosafety recommendations for laboratories conducting diagnostic testing for EVD without appropriate BSL3/BSL4 facilities

- Specimens for either PCR or ELISA testing should be processed inside a Class III biosafety cabinet (glovebox) with current certification in a separate laboratory area.
- Following inactivation, specimens can be removed from the glovebox and all other procedures performed under BSL2 conditions.
- Use appropriate Personal Protective Equipment (PPE) when handling the specimens before inactivation: gloves, fit-tested masks such as N95 Respirators and Filtering Face Piece (FFP) 3, Powered Air Purifying Respirators (PAPR) if fit-testing fails, full face shields, and disposable impermeable gowns.

Note: All liquid and solid wastes should be treated with care and undergo proper decontamination. Specimen containers and laboratory surfaces should be appropriately decontaminated.

3. Laboratory diagnosis

Laboratory results should be communicated to WHO as quickly as possible, in addition to International Health Regulations (IHR) reporting.

- For early detection of Ebola virus in suspect or probable cases, detection of viral RNA or viral antigen are the recommended tests.
- Laboratory-confirmed cases must test positive for the presence of the Ebola virus, either by detection of virus RNA by RT-PCR, and/or by detection of Ebola antigen by a specific Antigen detection test, and/or by detection of Immunoglobulin M (IgM) antibodies directed against Ebola.
- Two negative RT PCR test results, at least 48 hours apart, are required for a clinically asymptomatic patient to be discharged from hospital.

Note: It is recommended that the first 25 positive cases and 50 negative specimens detected by a country without a recognized national reference Viral Haemorrhagic Fever (VHF) laboratory should be sent to a WHO Collaborating Centre for VHF for secondary confirmation testing. Similarly, for countries with a national reference VHF laboratory, the initial positive cases should also be sent to a WHO Collaborating Centre for VHF for confirmation. If results are concordant, laboratory results reported from the national reference laboratory will be accepted by WHO.

4. Shipping specimens from cases under investigation for EVD

WHO has established an Ebola Shipment Funds Project with World Courier to facilitate shipments from countries to the WHO Collaborating Centres for VHF. For more information on this initiative, enquiries should be sent to edpln@who.int.

For countries without the capacity to test clinical specimens from cases under investigation:

- Clinical specimens should be shipped as Category A, using proper packaging, labelling, markings and documentation^v
- Prior discussion with the recipient laboratory is necessary to arrange timely shipment and processing of the specimen
- Specimen details must be provided to the recipient laboratory.

The following WHO Collaborating Centres for VHF have the capacity to confirm EVD:

- National Microbiology Laboratory Public Health Agency of Canada (Winnipeg, Canada)
- Institut Pasteur de Lyon (Lyon, France)
- Centre International de Recherches Médicales de Franceville (Franceville, Gabon)
- Bernhard-Nocht Institute for Tropical Medicine (Hamburg, Germany)
- Kenya Medical Research Institute (Nairobi, Kenya)
- Institut Pasteur de Dakar (Dakar, Senegal)
- National Institute for Communicable Diseases (Johannesburg, South Africa)
- Uganda Virus Research Institute (Entebbe, Uganda)
- Centers for Disease Control and Prevention (Atlanta, United States of America)

5. Occupational health

All laboratory personnel working with specimens suspected or confirmed of containing Ebola virus should immediately report any symptoms matching the case definition of EVDVI to health authorities and the head of their laboratory.

Incidents or accidents involving potential or actual exposure to Ebola virus should be immediately reported and any affected laboratory area/equipment appropriately decontaminated. Personnel who may have been exposed should seek medical advice as soon as possible.

6. Useful links

Please consult the WHO website (<http://www.who.int/csr/disease/ebola/en/>) for regular updates on all laboratory-related documents.

- *Ebola virus disease*. Geneva, World Health Organization, September 2014 (<http://www.who.int/mediacentre/factsheets/fs103/en/>)
- *In-Country shipment: How to safely ship human blood samples from suspected Ebola cases within a country by road, rail and sea*. Geneva, World Health Organization, 2014 (<http://www.who.int/csr/resources/publications/ebola/blood-shipment/en/>)
- *How to safely collect blood samples from persons suspected to be infected with highly infectious blood-borne pathogens*. Geneva, World Health Organization, 2014 (<http://who.int/csr/resources/publications/ebola/blood-collect-en.pdf>)

References

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- ⁱ *Case definition recommendations for Ebola or Marburg Virus Diseases*. Geneva, World Health Organization, 9 August 2009. (<http://www.who.int/csr/resources/publications/ebola/ebola-case-definition-contact-en.pdf?ua=1>)
- ⁱⁱ *Laboratory Biosafety Manual (3rd edition)*. Geneva, World Health Organization, 2004. (http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/)
- ⁱⁱⁱ *Ebola and Marburg virus disease epidemics: preparedness, alert, control, and evaluation: Interim manual version 1.2*. Geneva, World Health Organization, August 2014. (http://www.who.int/csr/disease/ebola/manual_EVD/en/)
- ^{iv} *Pathogen Safety Data Sheet – Infectious Substances*. Ottawa, Public Health Agency of Canada, August 2014. (<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/ebola-eng.php>)
- ^v *Guidance on regulations for the Transport of Infectious Substances 2013-2014*. Geneva, World Health Organization, 2012. (http://www.who.int/ihr/publications/who_hse_ihr_2012.12/en/)
- ^{vi} *Case definition recommendations for Ebola or Marburg Virus Diseases*. Geneva, World Health Organization, 9 August 2009. (<http://www.who.int/csr/resources/publications/ebola/ebola-case-definition-contact-en.pdf?ua=1>)