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# DIAGNOSIS AND HANDLING OF EBOLA VIRUS SAMPLES BEFORE AND AFTER TESTING-REVIEW

Dr.Noha Laj
Department of Microbiology
A J College of Science and Technology,
Thonnakkal, Trivandrum, Kerala, India

Dr.Raishy R Hussain
Department of Biotechnology
A J College of Science and Technology,
Trivandrum, Kerala, India

Abhinsha. Z
Department of Microbiology
A J College Thonnakkal
Trivandrum, Kerala, India

***Abstract*— EVD, a fatal disease is difficult to diagnose due to the similarity of the symptoms with many other diseases. This review article is an attempt to detail the diagnostic technique, collection of the specimens and the guidelines to be followed for transportation and the waste management of samples after testing.**

*Keywords*— **Ebola, diagnosis, sample collection, transportation, decontamination, disposal.**

## I. INTRODUCTION

EBOLA viral disease is often severe and causes fatal diseases and is transmitted mainly through contact with infected blood or body fluids or other objects such as needles that are contaminated with body fluids. Patients transmit Ebola virus after the onset of symptoms i.e. after 8-10 days, which is the incubation period. Those who encounter the criteria recommended by CDC, i.e. persons under investigation (PUIs) must be tested for Ebola.

## II. DIAGNOSIS

Diagnosing Ebola virus shortly after infection is a tedious process and requires great expertise. Diagnosis is usually done taking into account the early symptoms such as fever, headache etc which are not specific to Ebola virus infection and are also seen in the case of malaria or typhoid. For determining the possibility of EVD, a combination of symptoms and a possible exposure (contact with blood or body fluids directly or indirectly, contact with infected bat or non-human primates and semen from a recovered man) within 21 days of the onset of symptoms is suggested. If a person shows signs of EVD or has a doubt of exposure, the person should be isolated. Samples should be taken and tested for Ebola virus which can be detected in blood only after the symptoms begin and reaches a detectable level only after three days. A positive test indicates the confirmation of EVD and public health authorities will take necessary steps to contain the virus. The two major diagnosis involved during Ebola outbreak are laboratory testing and differential diagnosis.

Laboratory testing includes both non -specific diagnosis and specific diagnosis. The possible non -specific diagnosis includes a low platelet count (thrombocytopenia), initially decreased WBC or White Blood Cell count and then increased WBC count, abnormalities in blood clotting such as prolonged prothrombin time and bleeding time. Elevated levels of liver enzymes like alanine aminotransferase (ALT) and aspartate aminotransferase (AST) is a common characteristic of this disease (1). Specific diagnosis includes the use of electron microscopy to detect the filovirions which can be identified by their unique filamentous shape. The other specific diagnostic method includes isolation of the virus by cell culture and detection of its RNA (PCR) (2) or protein (ELISA) (3). Antibody detection in blood is also an efficient tool of diagnosis (3). IgM antibodies are detectable two days after the symptoms begin, but IgG can be detected only after 6 to18 days (2). The most common and reliable diagnostic measure during an outbreak of EVD is real time PCR and ELISA (4). A rapid antigen test which gives result in 15 minutes was approved by WHO (5) and is able to confirm about 925 of affected cases.

Differential diagnosis includes extensive consideration of many other infectious diseases also such as scrub typhus, sepsis, plague, measles, viral hepatitis to mention a few (6). Other non- infectious diseases that resemble the symptoms are Kawasaki disease, hemolytic uraemic syndrome, clotting factor, platelet deficiencies or disorders, warfarin poisoning, snake envenomation etc (4,7,8,9).

## III. COLLECTION, STORING AND TRANSPORTING OF SPECIMEN

Specimens should be obtained when a patient meets the criteria for person under investigation (PUI). In case the first specimen is tested negative (collected within 1-4 days after symptoms begin), a later specimen must be collected to dismiss Ebola viral disease infection. Staff should wear appropriate PPE before collecting specimens, care should be taken while Putting On (Donning) and Removing (Doffing).

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*A. Collection*

- A minimum volume of 4ml of blood should be taken from adults and 1ml from pediatrics, should be collected in small plastic collection tubes.
- Blood must not be transported in glass containers or in heparinized tubes.
- Blood should be preserved with EDTA and not with sodium polyanethol sulfonate, or clot activator or with citrate.
- Serum or plasma should do not be separated.
- Specimens should be packaged with 20C- 80C.

*B. Storing*

Under necessary conditions, the specimens should be stored at 4oC or frozen condition before storage.

*C. Transporting*

Ebola virus comes under Category A infectious substance and while transporting, specific guidelines have to be followed and transportation can be of two types- transporting within the facility and outside the facility. PPE kit which includes disposable fluid-resistant closed lab coat, gloves, covered legs and closed-toed shoes, must be worn throughout. Before removing patient specimens from the site of care, the route of the sample must be planned appropriately and the outside of the specimen container should be decontaminated.

*D. Transporting within the facility*

Specimens should be placed in a durable, leak-proof secondary container at par with OSHA Bloodborne Pathogens Standard.

The specimens should then be hand- carried to the laboratory or packing area. Avoid using pneumatic tube system for transporting specimens.

*E. Transporting outside the facility*

Specimens used for shipment must be packed following the triple packaging system containing,

•Primary container (sealable specimen container).

•Secondary container (watertight, leak-proof container).

•Outer shipping package.

Persons involved in the packing and shipping of infectious substances must be trained under IATA requirements every two years.

Specimens should be shipped without attempting to open collection tubes. Once opened, it may lead to destroying of the vacuum seal and thereby increasing the risk of leakage while transporting.

## IV. Decontamination

Disinfectants are known to kill no-developed viruses and thus can be used for cleaning and disinfection of testing surfaces, decontamination of laboratory equipment and handling spill.

*A. Cleaning and Disinfecting of Testing Surfaces*

PPE kit must be worn all the time while cleaning and disinfecting. In case the gloves used are reusable, they should be disinfected and kept in the anteroom. Always use EPA registered hospital disinfectant. Avoid contamination of reusable porous surface which are not for single use- curtains, carpets and furniture should not be used. Routine cleaning and disinfection of the PPE doffing area i.e. once per day. Reduce exposure among staff to potentially contaminated textiles (cloth products) while laundering, discard all linens, nonfluid-impermeable pillows or mattresses, and textile privacy curtains into the waste stream and dispose of appropriately.

*B. Handling spills*

While removing bulk spill material, cleaning the site and then disinfecting the site certain points are to be considered.
1.Protocols must be developed for safely remediating spills that contain broken glass.
2.Number of personnel involved in cleaning should be limited.
3.Staff should wear PPE including a minimum of disposable gloves, front wrap-around gowns (fluid impermeable), N-95 respirator, eye protection (full face shield or goggles) and should be trained before cleaning up is initiated.
4.The materials used for clean-up should be disposed of in a biohazard container.

*C. Decontamination of equipment*

Utmost care should be taken while decontaminating the equipment. Certain opinions for decontamination are: -
1.Use of EPA- registered hospital disinfectant for non-enveloped viruses is recommended.
2.Manufacturer must be contacted in advance to ensure the most appropriate selection of disinfectants, since some of them may be detrimental to the equipment.
3.Operator manual must be followed to see the recommendations while commissioning and preparing for maintenance or repairs.
4.In case of contamination of the equipment and no proper procedure is available for decontamination, then the instrument must be removed.

## V. Laboratory Waste Management

Since Ebola is considered as a Category A infectious substance, waste management must be strictly under the guidelines provided by the Laboratory. For solid waste generated during laboratory testing, the infectious material should be placed in a primary container that prevents leakage during collection, handling, processing etc. This primary container must be placed in a second container which is

puncture resistant. Autoclaving will inactivate the virus and thus after autoclaving, it can be combined with laboratory waste stream as regulated medical waste. In the case of lack of autoclaves, other arrangements with a licensed external waste contractor must be arranged to treat and dispose the waste. The regulations for waste management depends on various state and local requirements and hence waste management program must be incompliance with it.

## VI. INFECTION CONTROL

People caring the infected persons should wear protective clothing (10) which does not leave skin exposed. Persons handling contaminated objects also must take care (11). It is recommended that training should be given to medical personnel for proper use of suit up and removing of PPE and in addition a person who is appropriately trained should be watching each step of these procedures to ensure donning and doffing is done properly (12). The infected person should be in barrier isolation (10) and all the wastes should be properly disinfected.

Ebolaviruses can be eliminated with heating at 600C for 30-60 minutes. Lipid solvents such as alcohol -based products, detergents, sodium hypochlorite, calcium hypochlorite at appropriate concentrations may be used to disinfect surfaces (13, 7).

General public should be educated about the risk factors of infection and preventative methods should be introduced like regular hand washing using soap and water (14). Bushmeat, which is an important source of protein should be handled and cooked thoroughly before consumption (15). Maintenance of proper protective barrier while performing burial rituals is required (16).

Transportation crews must follow certain isolation procedure, in case anyone exhibit symptoms resembling EVD (17). In laboratories where tests are carried out, containment of BSL-4 is required and the researchers must be properly trained in it (18).

Control of outbreaks require rapid detection, isolation of the sick and contact tracing which is important to contain an outbreak. Contact tracing involves finding everyone who had close contact with the infected individuals and monitoring them, isolating them if tested positive and then tracing the contacts' contacts (19, 20).

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