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Revised NYS/NYC Laboratory Guidelines for Handling Specimens from Patients with Suspected or Confirmed Ebola Virus Disease

Purpose

The following revised guidelines are provided for New York State and New York City laboratories that may receive and test specimens from patients who are either:

- Suspected of having Ebola Virus Disease (EVD) and report low or high risk exposure[a] or
- Confirmed as having EVD with a laboratory test.

For patients with NO known exposures for EVD[b], specimens should be received, processed and tested in accordance with usual and standard procedures for laboratory testing.

For the purpose of these guidelines, a suspected EVD patient who reports either a Low or High Risk exposure[a], for whom a definitive diagnosis has not yet been determined, should be tested for Ebola virus after approval by both the local and state health departments as well as the Centers for Disease Control and Prevention (CDC).

Molecular EVD testing in NYS and NYC

Molecular diagnosis for EVD is available at both the NYC and NYS public health laboratories with a real-time RT-PCR assay that has been FDA-cleared under Emergency Use Authorization (EUA).

- Contact your local health department before collecting samples for testing, to obtain the **required prior approval for testing and assistance with specimen transportation.**
- For negative results on specimens collected less than 3 days post onset of symptoms, repeat testing is recommended unless a definitive alternative diagnosis has been made and EVD is no longer in the differential diagnosis.

EVD transmission and decontamination

Please note the following points with regard to EVD:

- A person infected with Ebola virus is not contagious until symptoms appear.
- EVD is transmitted through direct contact (via broken skin or mucous membranes) with blood or body fluids from an EVD patient, or through contact with objects contaminated with blood or body fluids from an EVD patient. There is no evidence of airborne transmission.
- Ebola virus is readily inactivated by standard chemical decontamination procedures used in laboratories.

Ebola virus is present in numerous body fluids of patients with EVD[1]. Although detected much less frequently, it has also been shown to be present in some environmental samples contaminated with blood or body fluid from an EVD patient consistent with a risk of transmission from fomites[1].

Biosafety classification

Two issues pertaining to Ebola virus biosafety classifications should be clarified. Information provided by the CDC (http://www.cdc.gov/vhf/ebola/hcp/safe-specimen-management.html) has verified that:

- While Ebola virus culture, which is commonly performed at high volume and can attain extremely high titer, is required to be performed at biosafety level 4, the handling of primary clinical specimens from EVD patients need not be restricted to this level of containment.
- According to the Interim Guidance Regarding Compliance with Select Agent Regulations for Laboratories Handling Patient Specimens that are Known or Suspected to Contain Ebola Virus, specimens from suspected EVD patients are **not** classified as select agents. For patients with confirmed EVD, select agent classification of specimens will be dependent on additional testing and consultation with the CDC.

CDC guidance

Guidance from the CDC recommends that suspected EVD patients who report Low or High Risk exposure, or laboratory confirmed cases, be managed in US hospitals with standard contact and droplet precautions. Laboratory personnel are advised to adhere strictly to safety procedures for the prevention of transmission of blood borne pathogens when handling specimens from these patients (www.cdc.gov/vhf/ebola/hcp/interim-guidance-specimen-collection-submission-patients--suspected-infection-ebola.html) including the following:

- Specimen collection
    - full face protection (mask and goggles or face shield), gloves, impermeable gown
- Laboratory testing
    - full face protection (mask and goggles or face shield), gloves, impermeable gown
    - use of certified class II Biosafety cabinet or splash shield

Note, the above guidance refers to all laboratory work including the routine hematology and clinical chemistry testing that is essential for the appropriate care and treatment of patients.

Supporting information

Information in support of these recommendations is provided below.

- Recent experiments in Canada have demonstrated the absence of airborne Ebola transmission in non-human primate experiments[2].
- An investigation of 173 contacts in 27 households demonstrated Ebola transmission only to those with direct physical contact or exposure to body fluids of the ill household member, and no transmission to the 78 household members who had no physical contact with the ill person[3].
- An investigation of three generations of Ebola transmission during an outbreak in Uganda, demonstrated direct contact with patient body fluids as the strongest risk factor for transmission, with contaminated fomites as a possible lesser risk factor[4].
- Several patients with viral hemorrhagic fever (VHF) have been cared for prior to being recognized as having VHFs in US and Western European medical facilities during the last several years. Although subsequently diagnosed as Lassa or Marburg fever, extensive follow up of hundreds of potentially exposed healthcare workers including laboratory personnel, have found no instances of transmission of infection[5,6,7,8].
- In 1996, a physician who had been working in West Africa and an anesthetics assistant previously involved in his care, became severely ill in Johannesburg, South Africa. Despite hospitalization for more than a week before being diagnosed with Ebola, and the performance of some potentially high risk medical procedures, none of the more than 300 exposed health-care workers, including laboratory personnel, contracted the virus[9].
- Lassa fever was detected in March/April 2014 in a patient in Minnesota with renal failure. The possibility of a VHF was not initially recognized and numerous health care workers including laboratory personnel were potentially exposed. However, there were no cases of disease transmission[10].
- Guidance documents from the UK note that one to two patients per year are diagnosed there with VHFs[11]. Some are not initially recognized as having VHF and are managed with standard precautions, yet there have been no reports of transmissions to health care workers. While VHF refers to a list of agents, not Ebola specifically, all are considered pathogens of “high consequence”.
- Reports in the literature of laboratory-acquired Ebola infections refer to events prior to the implementation of universal precautions and the availability of relevant safety devices such as retractable needles[12] or to infections acquired during the performance of animal necropsy and other animal experiments[13].
- On average, routine laboratory testing is performed on a few patients per year collectively at healthcare facilities in the UK, US and Europe. In some cases dozens of samples per case are processed and tested before the patient is diagnosed with VHF. Therefore collectively in these countries since the implementation of universal precautions approximately 30 years ago, it

would appear that hundreds of samples have been tested in laboratories using these procedures routinely, with no documented transmission to laboratory workers.
- To assist with the current outbreak in West Africa, laboratory personnel have been deployed to the European field laboratory in Guinea since mid-March, the Canadian field laboratory since June, and the two CDC laboratories since early August. Additionally, three other field laboratories set up by international partner groups are operational there. These laboratories process 200-300 specimens per day, yet there have been no documented cases of Ebola transmission to any of the laboratory scientists working at them. Earlier in the outbreak, some local West African laboratory personnel who were not wearing appropriate PPE and were performing procedures such as blood smear preparations without gloves, did acquire EVD. However, this has not occurred in any personnel wearing correct PPE and adhering to recommended procedures.

Nevertheless, Ebola virus is indisputably a highly pathogenic agent[14]. All laboratory directors should review their circumstances, facilities, resources and procedures, as well as the training and experience of their staff, in order to perform a thorough biohazard risk assessment and implement appropriate procedures for risk mitigation. However, any additional precautions or procedures should not interfere with the ability to provide appropriate medical care for suspected or confirmed EVD patients.

In light of all of the above, the following additional guidance is provided for consideration for the handling of laboratory specimens from suspected EVD patients reporting High or Low risk exposures or laboratory confirmed EVD cases.

General laboratory comments

- Laboratory testing should be limited to those tests essential to patient care. However, patient care and wellbeing should not be compromised.
- Specimens should be labeled to indicate that they have originated from a suspected High or Low risk, or confirmed EVD patient.
- Facilities should maintain a log of personnel handling specimens from these cases.
- Laboratories should review their protocols for occupational exposure and consult with their hospital epidemiologist and the local or state health department immediately if a potential exposure occurs.
- If available, the use of Point-of-Care instruments and methods inside or nearby the patient's isolation room may be a preferred option, to provide reduced specimen transport and limit the need for testing in routine laboratories.
- For testing that requires transport of samples to the hospital laboratory, specimens should be double-bagged, placed in a biohazard transportation container, and **hand-carried** to the laboratory. **DO NOT** use a pneumatic tube system.

Comments on specific laboratory procedures

Procedure	**Recommendation**
Centrifugation	Should be performed with sealed buckets or sealed rotor.
Homogenization	Procedures requiring homogenization of any specimen type should be avoided or performed with extreme care due to the risk of spray or splash.
Clinical chemistry and hematology	Numerous issues pertaining to routine testing in these areas need to be considered and are highly variable depending on the type of equipment used, volume of testing performed, laboratory workflow and layout, and many other factors. A full risk assessment should be made at each site, including options

	for decontamination[15]. For automated instruments, decontamination procedures should be those advised by the manufacturer or vendor for enveloped viruses.
Malaria testing	Rapid antigen tests or thin blood smears are preferred: recognizing that rapid tests are inherently less sensitive but positive results generally reliable. The effects of some inactivation/decontamination procedures on the performance of some rapid antigen tests for malaria have been investigated[16]. Thin blood smears should be fixed in methanol for 30 minutes and dried prior to staining. The use of additional heat inactivation is not considered necessary for Ebola decontamination and has been found by some parasitologists to cause disruption to the morphology of the parasites. Thick blood films are not recommended.
Blood Cultures	Systems using plastic blood culture bottles are preferred. Blood culture in glass bottles should be avoided.
Other specimens for bacterial culture	"Pan-cultures" should not be performed. Procedures essential for patient management should be performed in a BSC2 with PPE.
Wet preps	Should be avoided.
Viral cultures	**DO NOT perform viral culture**, including any rapid culture systems, on any specimen.
Pre-transfusion testing	Please refer to the American Association of Blood Banks' Ebola information sheet http://www.aabb.org/press/Pages/Infection-Control-for-Handling-Blood-Specimens-from-Suspected-Ebola-Patients.aspx
Post-mortem examinations	Should not be performed.
Specimen storage	With the exception of circumstances where retention is required by regulations, long-term storage of specimens is discouraged. It is recommended that specimens collected from suspected or confirmed EVD cases be isolated from other specimens in the laboratory and disposed of in an appropriate manner (see below) as soon as is practical after testing has been completed.
Specimen decontamination and disposal	Autoclave specimens if facilities are available. Alternatively, decontaminate specimens in 10% bleach for 24 hours, then place in standard biohazard infectious waste disposal.

This document has been prepared in consultation with more than 40 microbiology, clinical chemistry, and hematology laboratory directors, infectious disease clinicians, epidemiologists, and scientific specialists in VHF at the CDC. The NYSDOH and NYCDHMH wish to thank the many people who generously contributed their time for the consideration of these issues.

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[a] Suspected cases who meet the CDC criteria for Persons Under Investigation include i) travel within 21 days before illness onset to an EVD outbreak affected area (See **http://www.cdc.gov/vhf/ebola/resources/distribution-map-guinea-outbreak.html#areas** for the current list of affected areas; ii) fever (> 38.6 °C or 101.5 °F); and iii) compatible symptoms for EVD (e.g., severe headache, myalgia, vomiting, diarrhea, abdominal pain or unexplained hemorrhage).

High risk exposure is defined as either i) percutaneous, mucous membrane or direct skin contact with blood or body fluid from a confirmed or suspected EVD patient without appropriate personal protective equipment (PPE); ii) laboratory handling of body fluids from a confirmed or suspected EVD patient without appropriate PPE or biosafety precautions, or iii) participation in funeral rites which include direct exposures to human remains in the geographic area where outbreak is occurring without appropriate PPE.

Low risk exposures are defined as i) healthcare workers in facilities that have treated confirmed or suspected EVD patients or ii) household members or others with direct contact with a confirmed or suspected EVD patient.

[b] No known exposures are defined as residence or travel to an EVD affected area without either High or Low risk exposures.