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

This guidance is updated from the previous document issued on September 16, 2014 by the NYS and NYC departments of health, following the release of several new guidance documents from the CDC and the availability of additional information.

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 high or some risk of exposure[a] or
- Confirmed as having EVD with a laboratory test.

For patients with low (but not zero) risk (formerly termed "no known risk") or no identifiable risk of exposure 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 High or Some 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 (PHLs) 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, and if the patient is still symptomatic, repeat testing is recommended unless EVD is no longer in the differential diagnosis

Molecular EVD testing with other FDA-approved devices

The FDA has issued EUA approval for some commercially available EVD tests. All such tests carry the FDA provision that patient results obtained with these assays, including positive test results, should **not** be used for patient management decisions. Laboratories must ensure that concurrent with the use of any of these devices is the immediate submission of additional samples through the relevant health department for confirmatory testing. For additional information please see:

http://www.aphl.org/aphlprograms/preparedness-and-response/Documents/APHL-Guidance-for-Clinical-Laboratories-Using-FDA-Authorized-Assays-for-Ebola.pdf

EVD transmission and decontamination

Please note the following points with regard to EVD:

- A person infected with Ebola virus is not contagious before 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 and hospitals (detailed recommendations below).

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 High or Some 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. See the following two sites for more information:
www.cdc.gov/vhf/ebola/hcp/interim-guidance-specimen-collection-submission-patients--suspected-infection-ebola.html
and
http://www.cdc.gov/vhf/ebola/hcp/safe-specimen-management.html

Recommendations include the following:

- Specimen collection
  - gloves, water-resistant gowns, full face shield or goggles, and masks to cover all of nose and mouth. Additional PPE may be required in certain situations (http://www.cdc.gov/vhf/ebola/hcp/procedures-for-ppe.html)
- Laboratory testing
  - gloves, fluid-resistant or impermeable gowns, masks to cover all of nose and mouth, eye protection such as full face shield or goggles
  - use of certified class II Biosafety cabinet (BSC2) or Plexiglass splash guard if BSC2 not available
  - if neither a BSC2 nor Plexiglass splash guard are available, laboratorians should wear all of the above and in addition, a full face shield
  - manufacturer-installed safety features for instruments, that reduce the likelihood of exposure, should also be used

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 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 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 biohazard sealed buckets or sealed rotor. The buckets or rotor should be opened inside a BSC2.
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	Malaria antigen detection kits may assist with initial urgent assessment but must be recognized as being inherently less sensitive than smear microscopy or PCR, at least one of which must be performed as soon as possible. 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 15-30 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 parasite morphology. Thick blood films should not be hemolysed with water, but should be stained with Giemsa stain that includes Triton X-100 to inactivate Ebola virus. Validated malaria PCR assays that have been approved by the Clinical Laboratory Evaluation Program for clinical use may be used to detect malarial parasites. For more detailed guidance, see the CDC recommendations on Malaria testing for suspected Ebola patients at: http://www.cdc.gov/malaria/new_info/2014/malaria_ebola.htm
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 as described above. Identification or characterization of subsequently cultured bacteria or fungi, can be performed with standard precautions.
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 **only** be performed under the explicit recommendation of the CDC and with their guidance. In the event of a fatality in a suspected or confirmed EVD patient in New York City (NYC), the NYC Office of the Chief Medical Examiner (OCME) must be contacted immediately.The OCME will take custody of the decedent and make the final determination about disposition of the remains. Facilities outside of NYC should contact their coroner or medical examiner for further guidance on the procedure in their locality.
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. As soon as is practical after testing has been completed and it has been confirmed by the CDC or PHL that the samples are not needed for further evaluations, they should be disposed of in an appropriate manner (see below). Note – details of specimen decontamination and disposal should be documented for any samples from a confirmed EVD patient, or a PUI of unknown status. While the relevant division at CDC has agreed to not classify these as select agent samples, that classification being reserved for positive cultures, they do reserve the right to request information and confirmation of destruction/disposal.

Specimen decontamination and disposal	Wet a piece of gauze with a US Environmental Protection Agency (EPA)-registered hospital disinfectant with a label claim for a non-enveloped virus (e.g., norovirus, rotavirus, adenovirus, poliovirus) and wipe the outside of the specimen container. The gauze and the disinfected specimen container should then be placed in a plastic bag and packaged with other contaminated waste for appropriate disposal or autoclaving. A list of EPA-registered disinfectants can be found at: http://www.epa.gov/oppad001/list-l-ebola-virus.html **Note**: Bleach or acidic chemicals **must NOT be mixed with TRIzol or any other reagent containing guanidine isothiocyanate, nor should they be disposed of together** in the same container, as reactive compounds and toxic gases are formed if they interact.
Handling of regulated medical waste (RMW)	If sending RMW generated from the care of suspected or confirmed EVD patients off-site for treatment, please note the packaging requirements described in the US Department of Transportation (USDOT) emergency special permit. Information on the USDOT emergency special permit (DOT SP 16279) can be found at: http://phmsa.dot.gov/hazmat/transporting-infectious-substances. Your facility will need to confirm with the Department of Environmental Conservation (DEC) that your transporter has approval under the USDOT emergency special permit to transport untreated EVD RMW. Contact DEC by phone at (202) 366-4535 or by email at Specialpermits@dot.gov. If EVD RMW will be treated on-site by autoclaving, please note: autoclave facilities require approval from NYSDOH. For additional information see: http://www.health.ny.gov/diseases/communicable/ebola/docs/autoclave_guidelines.pdf For laboratory equipment that drains directly into the sewer system, discharge of liquid waste from equipment that drains directly into the sewer is allowed, unless specifically prohibited by local law or ordinance. Additional CDC guidance on RMW can be found at: http://www.cdc.gov/vhf/ebola/hcp/medical-waste-management.html and http://www.cdc.gov/vhf/ebola/hcp/interim-guidance-specimen-collection-submission-patients-suspected-infection-ebola.html

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[a] Suspected cases who meet CDC criteria for Persons Under Investigation include a person who has both consistent signs or symptoms and risk factors as follows: elevated body temperature or subjective fever or symptoms, including severe headache, fatigue, muscle pain, vomiting, diarrhea, abdominal pain, or unexplained hemorrhage; **AND** an epidemiologic risk (http://www.cdc.gov/vhf/ebola/exposure/risk-factors-when-evaluating-person-for-exposure.html) factor within the 21 days before the onset of symptoms.

High risk exposures include any of the following: i) Percutaneous (e.g., needle stick) or mucous membrane exposure to blood or body fluids of a person with Ebola while the person was symptomatic; ii) Exposure to the blood or body fluids (including but not limited to feces, saliva, sweat, urine, vomit, and semen) of a person with Ebola while the person was symptomatic without appropriate personal protective equipment (PPE) (http://www.cdc.gov/vhf/ebola/hcp/procedures-for-ppe.html); iii) Processing blood or body fluids of a person with Ebola while the person was symptomatic without appropriate PPE (http://www.cdc.gov/vhf/ebola/hcp/procedures-for-ppe.html) or standard biosafety precautions; iv) Direct contact with a dead body without appropriate PPE (http://www.cdc.gov/vhf/ebola/hcp/procedures-for-ppe.html) in a country with widespread Ebola virus transmission (http://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/distribution-map.html); v) Having lived in the immediate household and provided direct care to a person with Ebola while the person was symptomatic.

Some risk exposures include any of the following: i) in countries with widespread Ebola virus transmission (http://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/distribution-map.html): direct contact while using appropriate PPE (http://www.cdc.gov/vhf/ebola/hcp/procedures-for-ppe.html) with a person with Ebola while the person was symptomatic; ii) Close contact in households, healthcare facilities, or community settings with a person with Ebola while the person was symptomatic. Close contact is defined as being for a prolonged period of time while not wearing appropriate PPE (http://www.cdc.gov/vhf/ebola/hcp/procedures-for-ppe.html) within approximately 3 feet (1 meter) of a person with Ebola while the person was symptomatic.

[b] Low (but not zero) risk exposures include any of the following: i) having been in a country with widespread Ebola virus

transmission (http://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/distribution-map.html) within the past 21 days and having had no known exposures; ii) having brief direct contact (e.g., shaking hands), while not wearing appropriate PPE (http://www.cdc.gov/vhf/ebola/hcp/procedures-for-ppe.html), with a person with Ebola while the person was in the early stage of disease; iii) brief proximity, such as being in the same room for a brief period of time, with a person with Ebola while the person was symptomatic; iv) in countries without widespread Ebola virus transmission (http://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/distribution-map.html): direct contact while using appropriate PPE (http://www.cdc.gov/vhf/ebola/hcp/procedures-for-ppe.html) with a person with Ebola while the person was symptomatic; v) traveled on an aircraft with a person with Ebola while the person was symptomatic.

No identifiable risk includes i) contact with an asymptomatic person who had contact with person with Ebola; ii) contact with a person with Ebola before the person developed symptoms; iii) having been more than 21 days previously in a country with widespread Ebola virus transmission ((http://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/distribution-map.html); iv) having been in a country without widespread Ebola virus transmission (http://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/distribution-map.html) and not having any other exposures as defined above.