Abstract

The 2014 West African Ebola outbreak has spawned a chain of preparedness planning in the United States (U.S.). Although clinical and public health laboratories are hard at work preparing for the presentation of highly suspect patients, a number of issues have been identified that need to be addressed. Guidelines have been developed and distributed; procedures need to be revised and training needs to occur. It is imperative that public health laboratory systems support their clinical infrastructure. The purpose of this article is to share information the authors have gleaned from their research and involvement in the 2014 public health response.

Keywords
- Ebola Zaire
- Hemorrhagic Fever
- Clinical Lab
- Public Health Lab
- Outbreak

Introduction

Ebola virus was first identified in 1976. Over the last 30 years, outbreaks have sporadically occurred in Africa, mostly in isolated and rural areas. In March 2014, the Guinea Ministry of Health reported the current outbreak. This outbreak was later confirmed by the Institut Pasteur laboratory in Paris as Ebola Zaire, which currently remains active in the West African countries of Sierra Leone, Liberia, Nigeria, and Guinea. Biocontainment in the present outbreak appears to be a challenge. The World Health Organization’s (WHO) total count as of August 31, 2014 is over 3,707 cases and steadily climbing with mortality rates ranging from 50%-60% (CDC [a], 2014; WHO [a], 2014).

When two U.S. citizens on humanitarian missions aiding in the Ebola effort became infected with the virus, media coverage of their transport back to the U.S. for treatment increased awareness of the outbreak and created concern within the American public regarding risk of transmission. Clinical and public health laboratorians are beginning to triage and test patients with travel histories from locations within the current outbreak, and the “worried well.” Grave concerns have surfaced within the medical, scientific, and safety communities regarding work with biosafety level 4 (BSL-4) viruses in U.S. laboratories. Because some medical personnel are also concerned about their ability to properly package patient samples to transport for testing, the Centers for Disease Control and Prevention (CDC), state, and local health departments are presently developing guidance and training to create a level of comfort and understanding within the scientific community.

Taxonomy

The Genus Ebolavirus belongs to the Family Filoviridae. Ebola species include Bundibugyo, Taï Forest ebolavirus (Ivory Coast), Reston, Sudan, and Zaire (Formerly, 2008; Jahrling et al., 1996; Kuhn et al., 2010). The well-documented Ebola Reston outbreak in a U.S. primate colony in 1989 provided evidence of aerosol transmission in primates (Jahrling et al., 1996). Ebola Zaire has the highest mortality rates, reaching up to a 90% case fatality rate (CFR) (CDC [a], 2014).

Signs and Symptoms

Early Ebola symptoms are non-specific, mimicking many other African febrile illnesses. Symptoms include, but are not limited to, fever, headache, muscle pain, weakness, diarrhea, vomiting, loss of appetite, joint aches, abdominal pain, and hemorrhage (CDC [a], 2014; Dixon & Shafer, 2014; Formerly, 2008; PHAC, 2014). The severest forms cause hepatic damage and renal failure and include central nervous system (CNS) involvement (Dixon & Shafer, 2014).

Transmission

In the 2014 West African outbreak, most cases resulted from post-mortem preparation of bodies for internment or from direct exposure to body fluids, including blood, urine, sweat, tears, semen, saliva, breast milk, and feces (WHO [b], 2014). The virus remains infectious in body fluids after the patient’s recovery, and cases of sexual transmission from semen exposure have occurred weeks after recovery (Briand et al., 2014; CDC [a], 2014; Dixon & Shafer, 2014; Formerly, 2008; PHAC, 2014). The severest forms cause hepatic damage and renal failure and include central nervous system (CNS) involvement (Dixon & Shafer, 2014).
New England Journal of Medicine reported that more than 150 healthcare workers were infected in the affected countries, and over 80 died. (Briand et al., 2014). Although the actual number is unclear, some who died were identified as lab workers. Very little data are available identifying high-risk procedures and routes of transmission leading to lab-acquired infections and fatalities.

Occupational Health for Lab Exposures

Clinical and public health laboratories serving in a preparedness capacity must develop a plan to address laboratory worker exposure to the Ebola virus. If a laboratory exposure to Ebola occurs, it must be immediately reported to the institutional health and safety department, local public health department, and the CDC Division of Select Agents and Toxins (DSAT) using a CDC/U.S. Department of Agriculture (USDA) Form 3, available at www.selectagents.gov under “forms.” To date, the U.S. Food and Drug Administration (FDA) has not approved a treatment for the Ebola virus; currently, the treatment is supportive therapy. However, experimental vaccines are in various stages of development and approval. Monoclonal antibodies were used to treat the two U.S. cases of Ebola virus transported from Africa to Emory University Hospital in Atlanta, Georgia (CDC [a], 2014). The data supporting the efficacy, or lack of treatment efficacy, have not yet been published. Vital occupational safety and health information needs to be collected and carefully analyzed for all ill field laboratory and healthcare workers. Hopefully, these data will provide more information on high-risk procedures and routes of transmission.

Interpreting CDC Guidelines

The CDC Guidelines have undergone a number of changes since the beginning of the 2014 epidemic. Although initial guidance recommended the use of glass specimen containers, updated guidance recommends the use of plastic specimen containers (CDC [a], 2014). The objectives with Ebola virus are to collect the specimens in secure containers, refrain from opening them, and minimize the number of staff handling them. There have been a number of queries regarding laboratory containment of Ebola virus manipulations. The containment recommendations for Ebola virus vary significantly among countries, and questions regarding the appropriate personal protective equipment (PPE) for Ebola virus work have also been raised. The current CDC disinfection guidelines state that scientists should use an Environmental Protection Agency (EPA)-registered disinfectant for decontamination of work surfaces and instrumentation (CDC [a], 2014). The disinfectant used on environmental surfaces should inactivate enveloped viruses (e.g., Ebola) and non-enveloped viruses (e.g., norovirus, rotavirus, adenovirus, poliovirus) (CDC [b], 2014). Guidance for decontamination of automated hematology and clinical chemistry equipment in diagnostic laboratories has not been clear. The next iteration of the Biosafety in Microbiological and Biomedical Laboratories (BMBL) manual should address these safety concerns (U.S. IHS, 2009).

Specimen Collection

Clinical laboratory scientists who perform phlebotomy have expressed serious safety concerns regarding direct contact with patients infected with the Ebola virus and the sharps injury risks posed during venipuncture. Current CDC guidelines recommend the same droplet precautions that are used for pathogens transmitted via the respiratory route (CDC [a], 2014). These droplet precautions are instituted for contact within 3 feet of an infectious patient in healthcare settings (CDC, 2005). The CDC guidelines (CDC [a], 2014; CDC [c], 2014) recommend the following PPE for specimen collection and laboratory testing:

- Full-face shield or goggles
- Mask to cover the nose and mouth
- Gloves
- Fluid-resistant or impermeable gowns
- Additional PPE may be required in certain situations, including but not limited to:
  - Double gloving
  - Disposable shoe covers
  - Leg coverings

The guidance regarding the use of respiratory protection requires staff to be trained and fit tested annually using a NIOSH-certified respirator. Since the term “mask” in the above list leaves interpretation open to the use of a dust or surgical mask, the guidelines must be clarified to stipulate that the minimum level for respiratory protection is a National Institute for Occupations and Safety Health (NIOSH)-approved N95 fitted face-piece (CCR, 1974; CCR, 2009). Positive pressure powered air purifying respirators (PAPRs) can be used in place of the N95; however, most clinical laboratorians have limited or no access to PAPRs. In the public health setting, PAPRs are preferred for high-threat pathogens because they are more comfortable to work in for long periods of time than N95 respirators. Shoulder-length PAPR hoods also provide splash protection that is not provided by a surgical mask or N95 (CDC [a], 2014; Sheets et al., 2014).

Virologists work in the controlled environment of a laboratory where droplet exposure due to a patient vomiting, coughing, or sneezing does not occur, as it may in a healthcare setting (Sagripanti et al., 2010). However, Pike, Sulkin, and Schulze (1965) proved that many lab-acquired infections are the result of routine manipulations of dangerous pathogens. This is of particular concern when working with high-consequence pathogens, such as Ebola, with a low infectious dose, and needs to be addressed.

Transport within the Hospital

After collection, a pneumatic tube system should never be used to transport specimens to the laboratory (CDC [e], 2014). Ensure that gasket sealed leakproof containers are used for transport within the laboratory (CDC [e], 2014). Double bagging the primary container can be used if a gasket sealed carrier is not available.
Shipping Specimens

Initial CDC packaging and shipping guidelines recommended that the submitter ship specimens directly to the CDC using the Category B requirements. However, revised classification guidelines include recommendations from the UN2814 Infectious Substance Affecting Humans list, which is in line with the National Select Agent Registry (NSAR) guidelines (CDC [d], 2014), Category A indicative lists from the International Air Transport Association (IATA, 2011), and the U.S. Department of Transportation (U.S. DOT, 2006). All three agencies advise or require using “suspected Category A infectious substance” as the technical name on documentation accompanying packages containing patient specimens or cultures known or suspected to contain Ebola virus. Laboratories that package and ship division 6.2 infectious substances are subject to DOT inspections. If the shipper does not have the proper training and certification as required by 49 CFR Parts §§ 172.700, 172.702, and 172.704, the laboratory can be cited.

Training to package and ship a Category A infectious substance includes, but is not limited to:

- Classifying the patient specimens and cultures properly.
- Properly packaging the Category A material in Class 6.2 UN specification packaging following both transportation regulations and packaging manufacturer information.
- Affixing the correct markings and labels to the package appropriately.
- Properly completing documentation that includes an itemized list of contents and the shipper’s declaration form.
- Providing emergency response information as appropriate for the mode of transport.
- Keeping transport records for 2 years (CDC, 2012; IATA, 2011; U.S. DOT, 2006).

One of the biggest challenges has been the classification of patient specimens for shipment. Health departments across the nation have worked diligently to prepare guidelines and answer questions from clinical and public health laboratories that are concerned about the appropriateness of shipping patient specimens as Category A infectious substances. The IATA (IATA, 2011), and DOT requirements (U.S. DOT, 2006) stipulate that if the presence of Ebola virus in a patient specimen is known or suspected, the package must be shipped as Category A, a classification that is determined in consultation with local, state, and federal public health partners. The following guidelines determine the classification of patient specimens before transporting for Ebola testing.

Classify as Category A if the patient has the appropriate symptoms and travel history, and fits the case definition criteria. These include:

- Clinical and laboratory features strongly suggest for Ebola virus infection.
- Direct contact is known or suspected with a confirmed or symptomatic case.
- Casual contact is known or suspected with a confirmed or symptomatic case.

Classify as Category B if the person has a travel history to an affected country, but the consultation with public health determines that:

- The clinical and laboratory features DO NOT suggest Ebola virus infection.
- NO direct contact is known or suspected with a confirmed or symptomatic case.
- NO casual contact is known or suspected with a confirmed or symptomatic case (CDC [c], 2014; CDPH DCDC, 2014; Sheets et al., 2014).

If the primary test request(s) from treating physicians and public health medical officer is for febrile illness other than a viral hemorrhagic fever (i.e., malaria, yellow fever, dengue, etc.), the specimen can be shipped Category B.

The logistics of shipping a Category A Risk Group 4 pathogen are challenging. Not all cargo and passenger airlines in the U.S. will transport Category A infectious substances. As a matter of convenience, most laboratories use cargo airlines to pick up and deliver packages directly to the testing lab. Most laboratories have contracts with either United Parcel Service (UPS) or Federal Express (FedEx). However, UPS and Dalsey, Hillblom, and Lynn (DHL) Express will not accept Category A infectious substances for transport. FedEx will ship Category A infectious substances, such as Bacillus anthracis, Brucella spp., Francisella tularensis, and Yersinia pestis, but will not ship a Category A risk group 4 infectious substance, which includes Ebola virus (IATA, 2011). In the U.S., World Courier is the only commercial cargo carrier that will ship Category A risk group 4 agents, but it is not available in all geographic regions. Therefore, the logistics of shipping a Category A risk group 4 pathogen are challenging.

If U.S. clinical laboratories follow the CDC guidelines that suggest consultation with public health partners on classification of patient specimens with unknown viral infections, the number of specimens sent for Ebola testing is expected to be very small. Nevertheless, laboratories must have employees who are trained prior to packaging any Category A infectious substance. Some labs that routinely ship only Category B infectious substances may not have considered having to be prepared for the rare specimen or culture that might require Category A packaging. In addition, many of the labs that do have trained employees lack confidence in their training to properly package, while others have had problems finding couriers and cargo airlines willing to carry Category A infectious substances. Thus, public health laboratories were asked to provide onsite specimen packaging and subsequent transport to their testing facilities for shipping. Public health laboratories have reached out to help clinical laboratories package, ship, and transport specimens.

Laboratory Containment and Safety Practices

There has been a lot of confusion regarding guidance for field, clinical, and public health laboratories concerning containment and safety practices with the Ebola virus. In the U.S., laboratory work with the Ebola virus is heavily regulated and must be done in a high-containment laboratory, under ideal conditions, and with properly trained staff wearing PPE. However, high-containment laboratory re-
search is not subject to the same resource constraints that field operations encounter. Field operations must be conducted with a number of limitations such as the absence of negative air flow, access to autoclaves, and limited electricity to charge PAPR batteries. Careful consideration must be given to field operations in order to conduct the manipulations in the safest way possible given the resources available. The BMBL 5th edition lists Ebola virus as a BSL-4 virus (U.S. HHS, 2009). The CDC Division of Select Agents and Toxins (DSAT) evaluates laboratories registered for Ebola virus for BSL-4 compliance (CDC [d], 2014; IATA, 2011; U.S. DOT, 2006). Culturing Ebola virus should be attempted only in a containment level 4 laboratory (PHAC, 2014; U.S. HHS, 2009). However, this year the CDC Laboratory Response Network (LRN) distributed an Ebola PCR assay with a protocol stating that extractions should be performed in a BSL-3 laboratory in a biosafety cabinet (BSC) with the appropriate PPE. Public health laboratories performing the LRN PCR, should read “Virus inactivation by nucleic acid extraction reagents” (Blow et al., 2004). The directions issued to clinical laboratories (e.g., hospitals, point-of-care testing) state that routine hematology and clinical chemistry tests can be performed at BSL-2 with enhanced PPE (BSL-2+) in a Class II BSC or behind a Plexiglas shield. This guidance contradicts the risk group 3 and 4 designations issued by CDC DSAT. Using a BSL-4 laboratory for viral culture makes sense. A negative pressure laboratory (BSL-3), with the appropriate combination of PPE, should be appropriate for extractions performed in a BSC. However, the manipulation of an EDTA or serum tube in the clinical lab setting, should be questioned. The next edition of the BMBL must clarify why Ebola extractions should be performed under BSL-3 conditions, but performing manipulations using automatic analyzers is acceptable under BSL-2 conditions.

A Plexiglas shield supplies splash protection but does not offer the negative pressure and HEPA filtration that the BSC does. They are not comparable engineering controls. The CDC guidance for clinical labs performing routine complete blood count (CBC) tests and clinical chemistry on a high-risk suspect Ebola patient is limited. Many hospital labs are refusing to perform routine diagnostic tests until a negative Ebola test result is received on suspect patients. Before routine diagnostic tests are conducted, a thorough risk assessment must be performed. If routine diagnostic tests are absolutely necessary, the ideal situation would be to perform the diagnostic testing in the patient’s negative pressure isolation room. The American Society for Microbiology (ASM) Guidelines recommend using 1-STAT or point-of-care (POC) testing systems for hemorrhagic fever viruses (HFV) (ASM, 2014). If an automated analyzer is used, a dedicated closed system is preferred. Laboratorians must review the mechanisms by which their automated analyzer(s) work, and ask the following questions when conducting the risk assessment: Does this require opening of an EDTA or serum tube? Are centrifugation steps part of the procedure? Is this an open system? Are there sample probes that move quickly with aerosol-generating potential? Does the analyzer probe use a vacuum to withdraw the sample from the tube? Is there aerosol-generating potential from the vacuum suction? How is the effluent considered infectious? How is an automated analyzer decontaminated? Is point-of-care testing possible in the room? Consider how slides will be handled and fixed to rule out similar infections such as malaria, if those are requested to be performed before Ebola is ruled out. Remember, the objective is to minimally handle the diagnostic specimens as safely as possible.

The 2005 and 2014 CDC guidelines state that specimens should be processed in a Class II BSC following BSL-3 practices. However, many clinical labs do not have a BSC or the recommended PPE available, and laboratorians may not be familiar with BSL-2+, or BSL-2 enhanced, procedures. The first time a scientist performs a procedure, he or she is likely to have a higher margin of error because of unfamiliarity with the required PPE and engineering controls. Review of the Hematology section of the “Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories” should help answer some of these questions (CDC, 2012). Review the 2014 CDC Ebola guidelines closely for updates (CDC [a] 2014; CDC [c] 2014; Fernstrom & Goldblatt, 2014).

CDC DSAT Compliance

CDC DSAT regulates Ebola virus work under 42 CFR part 73, designating the virus as risk group 4. A CDC Select Agent Gram (SA GRAM) was distributed on August 20, 2014 confirming the designation of this particular Ebola manipulation as risk group 3, allowing extractions for the LRN PCR to take place in a BSL-3 laboratory (CDC [f], 2014). If diagnostic specimens from high-risk patients are submitted to the CDC for Ebola confirmation, and they test positive, per the regulations, any retained specimens must be transferred or destroyed within 7 days of receiving confirmation. The confirmatory reference lab will require the submitter to complete the second page of the CDC/USDA Form 4. The official transfer process is quite cumbersome, but can be initiated using a CDC/USDA Form 2, available at www.selectagents.gov under “forms.”

Medical Waste Management

The preferred method for waste decontamination prior to disposal is to autoclave, with incineration as another option. However, most labs don’t have access to an incinerator and local legislation may impose restrictions impeding the use of incinerators. The most recent CDC guidelines recommended following the medical waste management requirements, which are unique to each state (CDPH, 2007). The guidelines also discuss equipment that drains in the sink. CDC suggested use of anaerobic digestion, composting, and disinfection for drain disposal (CDC, 2014e). The caveat is that drain disposal varies by jurisdiction. Be certain all work complies with local and state jurisdictional requirements. The guidance also states that diagnostic Ebola specimens are medical waste covered under the exclusion 42 CFR part 73.3(d)(1).
California ATD Standard

California has unique requirements for aerosol-generating procedures. Any manipulation that imparts energy onto a patient specimen, thus creating a droplet spray or aerosol, is regulated by the California Aerosol Transmissible Diseases (ATD) standard. Appendix D of the California Code of Regulations (CCR) Title 8 Section 5199 regulates droplet or aerosol-generating procedures using Ebola virus. The current guidelines recommend the use of standard droplet precautions. Is a surgical mask with face shield adequate for the collection of a risk group 4 agent with a low infectious dose? In California, a surgical mask is not considered adequate for aerosol-generating procedures (i.e., vortexing, centrifugation) or automated devices (vacuum suction) that may lead to the production of aerosols in the laboratory (Fernstrom & Goldblatt, 2014). Laboratories should adhere strictly to aerosol precautions for aerosol-generating procedures. Laboratories conducting procedures with Ebola virus generating infectious aerosols must utilize the appropriate PPE, engineering controls, and safety practices. These decisions should be made in consultation with a qualified biosafety officer performing a thorough risk assessment in accordance with the current BMBL as described in CCR Title 8 Section 5199. Please note that all applicable federal, state, and local public health guidelines also apply.

Conclusions

A number of lessons have been learned from the 2014 U.S. Ebola response. Based on experience, the authors recommend the following:

- Consult with a public health and biosafety officer when conducting your risk assessment.
- Ensure compliance with all federal and state statutes related to clinical standards, occupational health, and shipping. Communicate with scientists performing the new assays early to research and address their safety concerns.
- Educate all staff when dealing with a public health emergency in emerging, or re-emerging, infectious disease situations.
- Assure that staff are certified to package and transport Category A infectious substances.
- Identify air carriers readily available to transport risk group 4 pathogens.
- Review packaging, shipping, and receiving procedures before packaging specimens.
- Contact shipping vendors to determine which will accept them.
- Practice new or updated procedures before beginning work with a high-threat pathogen. Good technique is important.
- Evaluate the competency of the scientists performing the procedure.

Laboratories across the U.S. are fortunate that all of the rule-out cases have been negative. This has given the public health system time to develop and test its response strategies. Scientists should take full advantage of this opportunity to prepare for a highly suspect Ebola case. Such planning will also be useful for other viral hemorrhagic fevers as well.

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References

Centers for Disease Control and Prevention (CDC). Interim guidance for managing patients with suspected viral


