Special Report

An Integrated Approach to Laboratory Testing for Patients with Ebola Virus Disease

Peter C. Iwen, PhD, D(ABMM),[1,3] Jodi L. Garrett, MT(ASCP)SM,[4] Shawn G. Gibbs, PhD,[2] John J. Lowe, PhD,[2] Vicki L. Herrera, MS,[3] Anthony R. Sambol, MA,[3] Karen Stiles, MT(ASCP)SM^{CM},[3] James L. Wisecarver, MD, PhD,[1,4] Kathryn J. Salerno, MT(ASCP),[4] Samuel J. Pirruccello, MD,[1,4] Steven H. Hinrichs, MD.[1,4]*

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Beginning in 2003, the Nebraska Medical Center in Omaha developed a laboratory capability plan in conjunction with the creation of a biocontainment unit (BCU) for treatment of patients harboring emerging infectious organisms. The laboratory response planning involved experts at the Nebraska Public Health Laboratory (NPHL), University of Nebraska Medical Center (UNMC), the Nebraska Department of Health and Human Services (DHHS), and the Centers for Disease Control and Prevention (CDC). Special emphasis was placed on diagnostic testing for highly contagious and pathogenic organisms, including *Francisella tularensis* and high consequence viruses causing avian influenza and hemorrhagic fevers such as Ebola.

Due to the recognition that certain organisms and conditions would need to be ruled out, preparations also included the capability to test specimens for other diseases, including malaria and tuberculosis. Originally, a limited number of point of care (POC) hematology and chemistry tests were planned, to monitor patients who harbored a high consequence pathogen. This testing was to be performed in the biosafety level 3 (BSL-3) laboratory within the NPHL at UNMC, which is within 1 city block from the Nebraska Medical Center, the main campus facility for the parent organization; the BCU is located at the Nebraska Medical Center. At various times, the laboratory staff conducted drills or participated in simulated training exercises with the medical staff of the BCU and state and national organizations to refine operational plans. Preparedness efforts included drafting of memoranda of understanding that detailed specific responsibilities and capabilities. However, when the first patient with Ebola virus disease (EVD) arrived, a number of factors resulted in reconsideration of the original testing plan.

As the critical nature of the patient's condition became clearer, the expectation from our clinical team was that optimal patient care required that an expanded list of tests be provided. An additional expectation was that the test results would be available within the same timeframe as those for other critically ill patients receiving intensive care. In particular, management of significant electrolyte imbalance and complications required more rapid turnaround time than originally proposed on a number of assays. Within our clinical team, we undertook an extensive review of laboratory support for patients with

Abbreviations

BCU, biocontainment unit; NPHL, Nebraska Public Health Laboratory; UNMC, University of Nebraska Medical Center; DHHS, Department of Health and Human Services; CDC, Centers for Disease Control and Prevention; POC, point of care; BSL-3, biosafety level 3; EVD, Ebola virus disease; HIV, human immunodeficiency virus; BSL-2, biosafety level 2; DoD, Department of Defense; EUA, Emergency Use Authorization; PPE, personal protective equipment

Keywords

Ebola virus disease, diagnostic laboratory testing, biocontainment, risk assessment

[1]Department of Pathology and Microbiology, College of Medicine

[2]Department of Environmental, Agricultural, and Occupational Health, College of Public Health, University of Nebraska Medical Center, Omaha, Nebraska

[3]Nebraska Public Health Laboratory, Omaha, Nebraska

[4]The Nebraska Medical Center, Clinical Laboratory, Omaha, Nebraska (now known as Nebraska Medicine)

*To whom correspondence should be addressed. piwen@unmc.edu

Ebola infection and we developed a consensus list of assays based on specific interventions and criteria.

The extensive nature of the requested assays required us to reevaluate our options. We considered performing all of the requested assays within the BCU, using an approach similar to that used by the laboratory located within the patient care containment area at Emory University in Atlanta, GA[1]; however, the requirement for rapid validation and 24-hour access did not permit that solution. One consideration was the CDC recommendation that Ebola virus testing could be handled in a clinical laboratory using standard precautions following risk assessment.[2] Our primary concern related to microdroplet generation by centrifuges and automated instruments, breakage of tubes and bottles during processing, and laboratory accidents. We investigated the quantity of blood potentially aerosolized by testing instruments. Because individuals infected with Ebola virus may have a viral genome titer of 1E8 or greater, if only 1 of 10 genomes were infectious, a nanoliter of tainted blood would deliver an infectious dose to mucous membranes and the eyes.

We consulted industrial hygienists, who evaluated our processes and instruments. All instruments were assessed, with a focus on relative risk for generation of microdroplets from instrument processes. The hygienists were aware that, before sale by the manufacturer, the laboratory instruments had not been certified as safe for use in the testing of specimens known to contain highly infectious organisms. However, our laboratory was aware that a significant number of specimens from patients infected with hepatitis C virus or HIV had been processed in the past with these instruments. The hygienists determined that certain instruments, such as the coagulation analyzer, represent a higher risk due to splatter created when testing cuvettes are discarded within the analyzer. It was also noted that the centrifuges on the automated lines did not use capped cups or have sealed rotor lids. In addition, it was found that performance of several requested assays on automated instruments would require decapping of the specimen. However, we identified certain instruments, including the Beckman Coulter DxI800 and DxC880i (Beckman Coulter Inc., Brea, CA) and the Sysmex XN 9000 with automation line (Sysmex Corporation, Kobe, Japan), as having closed-tube systems that could be operated under biosafety level 2 (BSL-2) conditions with a favorable level of safety.

The results of the overall assessment and our experience with other highly infectious organisms resulted in a revised process. This new process not only took advantage of the capabilities of the NPHL BSL-3 laboratory suite but also the core laboratory of the Nebraska Medical Center. It was determined that the process of decontamination of the specimen and transportation to the NPHL BSL-3 resulted in delay of testing for up to an hour. Therefore a new laboratory was developed within the BCU.

An expanded menu of tests was developed for performance in the BCU BSL-3. For purposes of rapid turnaround, our preference was to perform testing in the BCU laboratory with POC instruments. Additionally, we transferred some of the manual, kit-based assays originally performed in the NPHL to the BCU BSL-3 laboratory. All assays were validated according to CLIA standards. It was determined that all centrifugation and all open tube testing would be performed in the BCU laboratory or the NPHL BSL-3 laboratory. Transportation of specimens required special attention, and the processes used to address this issue are summarized in a separate section herein.

Processes and Testing Performed in the BCU BSL-3 Laboratory

Centrifugation of Blood for the BCU or Core Laboratory.

iSTAT 1 analyzer (Abbott Laboratories, Abbott Park, IL): G3+ cartridge (arterial/venous blood gases), Chem 8+ cartridge (electrolytes, chemistries, and hematocrit).

ITC Hemochron Signature Elite (citrate prothrombin time (PT)/citrate-activated partial thromboplastin time (aPTT).

Blood and serum antibody typing (using slide agglutination).

Malaria testing: modified for the slide to be fixed in methanol for 15 minutes before delivery to the Core Laboratory for staining and interpretation.

Urine chemistry: for determinations not available by dipstick, BCU personnel transferred patient urine specimen(s) under a BSC into the BD Vacutainer urinalysis plus urine tube (Beckton, Dickinson, and Company, Franklin Lakes, NJ) for transportation to the core laboratory and performed testing on the Beckman Coulter Unicel DxI800 and DxC880i without decapping specimens.

HIV Ab/Ag: rapid manual assay.

Syphilis rapid plasma reagin (RPR) testing: card assay.

Processes and Assays Available in the NPHL BSL-3 Laboratory

Centrifugation: performed as needed for assays in the NPHL or for testing at the CDC.

CDC/Department of Defense (DoD) EZ1 Emergency Use Authorization (EUA) Ebola assay: As of the writing of this article, this test requires prior approval by the CDC for its use in patients under investigation for Ebola virus disease. It is commonly referred to as the Ebola screening assay. It is currently available at 18 public-health laboratories around the country.

Biofire FilmArray Biothreat Panel (BioFire Diagnostics, Inc., Salt Lake City, UT): this instrument panel was available within the laboratory; however, we obtained the reagents for the Ebola assay for investigational purposes only. As of the writing of this article, the Biofire FilmArray assay is not approved by the United States Food and Drug Administration (FDA), and EUA status for this assay is pending.

Blood cultures: plastic blood-culture bottles were used with stationary incubation and daily plating to media.

Specimen repository: personnel from the NPHL recovered all tubes and specimens submitted for testing. They also processed, logged and, when appropriate, packaged all specimens for transport to the CDC or other entities for research protocols related to drug studies.

Procedures and tests performed by the core laboratory of the hospital

Testing was only performed on instruments with closed-tube sampling capability in the core laboratory. No opening and/or decapping of tubes was allowed outside the BSL-3 laboratory.

The DxI, DxC, and Sysmex hematology analyzer provided 24-hour access to a broad list of assays to address the clinical need for rapid turnaround time of such analytes as chemistry panels, complete blood counts, cortisol, lactate, and magnesium. Regarding DxI testing, to address the generation of a specimen aliquot by the analyzer, a biohazard sticker was placed on the DxI solid waste container prior to testing. After testing, the DxI was initialized to clear the used reaction vessel and this waste was removed by the industrial hygienist and disposed following BCU protocol. Testing of specimens from other patients was not stopped or disrupted.

Malaria smears were prepared in the BCU BSL-3 laboratory by methanol fixation for 15 minutes and then transported to the core laboratory. Malaria smear interpretation was performed within the core laboratory to take advantage of the skills of the experienced hematology technologists.

Once testing on a specimen was completed, the specimen was placed in the transport carrier for return to the NPHL for storage. NPHL personnel were responsible for packaging the specimen for testing at the CDC (Ebola confirmation and quantification) or for experimental studies. Instrument racks were decontaminated with bleach after testing within the core laboratory.

As noted in the CDC guidelines, a separate assessment of all operating procedures, facilities, and equipment was completed. The team of industrial hygienists proved very helpful in the initial assessment and the continuous monitoring of risk. They also provided support for decontamination of waste within our facility and transport to offsite facilities.

Transportation of Specimens Within the Hospital or on Campus

Before removal of specimens from the BCU, external surfaces were decontaminated, and the specimens were triple bagged. The bags were placed in a hard-sided container and escorted by 2 people. We required 2 escorts for this part of the process to ensure specimen security. We did not allow transportation of specimens using the pneumatic tube system. We performed all centrifugation in the BCU laboratory unless the test was to be performed in the NPHL or sent to the CDC, in which case the centrifugation occurred in the NPHL BSL-3 laboratory.

When specimens were ready for transport, a technologist within the BCU laboratory called the core laboratory for transportation services. Laboratory personnel were paired with a campus security officer and then transported the specimen in a special carrier to the laboratory, where it was handed directly to a core laboratory technologist. None of the specimens were taken to the standard specimen receiving area.

Transportation of Specimens Outside the Institution (ie, to the CDC)

The shipping of specimens outside our medical facility for testing at the CDC was required to evaluate specimens from individuals under investigation for EVD from our facility or the region. In all cases involving the evaluation of specimens from a patient suspected of having EVD, we had first made contact with state and/or local public health department officials. For patients undergoing therapy, we sent additional specimens to the CDC for evaluation of response. All specimens for EVD testing underwent a review process before transport.

We found that the regulations related to shipping were very specific and required special knowledge. According to federal regulations, specimens that are confirmed positive for Ebola virus by culture methods are considered select agents, whereas specimens that test positive by molecular assays are considered category A agents, not select agents.[3] In addition, if the body of a patient has yielded a specimen found to be positive by culture, all subsequent specimens are also affected and are automatically classified as select agents. We found the September 8, 2014 CDC document "Interim Guidance Regarding Compliance with Select Agent Regulations for Laboratories Handling Patient Specimens that are Known or Suspected to Contain Ebola Virus"[3] to be helpful in this regard. Due to the complexity of this regulation, special efforts were made to educate all medical personnel regarding the consequences of a researcher or other institutions performing culture procedures on any specimens from individuals with EVD.

All specimens from individuals suspected of being infected with Ebola virus were shipped as a category A package. We discovered that certain commercial shippers, such as FedEx Corporation (Memphis, TN), would provide the transportation. Specimens not confirmed by culture but which tested positive by molecular assay were transported with category A packaging as "suspected category A infectious substances." In this case, such specimens could only be transported by a limited number of certified couriers who accept category A risk group 4 pathogens. We hired World Courier (AmeriSource Bergen Corporation, Chesterbrook, PA) to provide this service because other commercial vendors would not transport a specimen that had tested positive for Ebola via molecular assay. Only employees who had been trained and certified to as category A shippers were allowed to package, mark, label, or complete the documentation for packages being offered to shippers for Ebola testing.

Patient specimens from individuals who were suspected to be infected with the Ebola virus and which were officially accepted for shipping to the CDC were properly packed in United Nations specific packaging according to International Air Transport Association (IATA) instruction 620 before being delivered to the shipper for transport. A declaration form from the shipper accompanied those packages, with the terms "suspected category A infectious substance" as the technical name on the declaration form from the shipper. We did not put the technical name on the outer packaging and only placed a premade label reading "class 9, infectious substance, affecting humans" on the external packaging.

Summary and Conclusion

Overall, we found that advanced preparation was the key to establishing a firm foundation on which to implement changes that were required as the situation evolved. An essential part of the training exercises was learning how to adjust to changing conditions. Familiarity among all the medical staff was particularly helpful in building trust and a cooperative environment. Risk assessment of our laboratory environment and our instrumentation was critical to maintaining a safe working environment. A research opportunity exists for the rigorous assessment of aerosols generated by automated laboratory instrumentation followed by recommendations to commercial vendors or regulatory agencies. The procedures that we developed resulted in a process that may adaptable to many clinical laboratories that operate a BSL-3 facility but do not have an in-hospital biocontainment facility. We concluded that following a risk assessment and mitigation strategy that specimens from patients confirmed positive for Ebola virus

can be safely handled and tested in the clinical laboratory using an integrated approach.

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Financial and Personal Conflicts of Interest

None reported.

Disclaimer

This article represents the opinions and observations of the authors and is not official policy of any government entity or the University of Nebraska Medical Center or Nebraska Medicine. Hospitals and laboratory professionals are responsible for development of their own procedures and operational plans related to preparedness for Ebola virus disease in compliance with state, local, and federal regulations. Resources and guidelines on Ebola virus disease management issues should be consulted as provided by the Centers for Disease Control and Prevention, the US Department of Transportation and the World Health Organization.

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Questions and Answers

Q: Who collects blood and urine specimens in your health care system?
A: Blood and urine specimens are collected by the registered nurse (RN) caring for the patient.

Q: How many technologists staff the biocontainment (BCU) laboratory?
A: There are always 2 laboratory professionals present when laboratory testing is being performed in the BCU laboratory to assure proper personal protective equipment (PPE) and, as needed, to hand supplies to the individual performing testing within the biosafety cabinet.

Q: What are your general rules for testing?
A: In the core clinical laboratory, we will not centrifuge or decap specimens containing or under suspicion for containing the Ebola virus.

Q: Do you perform dilutions for specimens that are above the standard ranges?
A: We do not perform any dilutions; our results are reported out as > linearity.

Q: Where do you perform blood gases?
A: Blood gases assays are performed using point of care (POC) testing in the BCU laboratory.

Q: What blood transfusion services or procedures do you use?
A: ABO RH testing is performed in the BCU laboratory using the slide agglutination method; no other blood bank testing is offered. In consideration of a hemolytic reaction, type O, Kell-negative blood is maintained.

Q: How do you handle laboratory testing of specimens from patients suspected of being infected with Ebola but whose diagnosis is not confirmed?

A: This is a complex issue. First, the decision depends primarily on the assessment of the emergency department or infectious diseases physicians. The second issue is that the Centers for Disease Control and Prevention (CDC) must give approval for use of the Ebola screening assay, which is available through 18 different public health laboratories around the country. The assay takes between 4 and 6 hours to perform; therefore, the clinical assessment is important. Sending a specimen to the CDC for confirmation may require 1 to 2 days, including transport time. Therefore, the general approach is to place patients in isolation if the history and clinical findings are consistent with Ebola and to provide supportive care to them. If additional testing were determined to be essential in consultation with our clinical colleagues, we would process the specimen in the biosafety level 3 (BSL-3) facility and would perform testing as if the patient were infected with the Ebola virus.

Q: How do you handle instrument downtime and/or equipment malfunction in terms of safety?
A: Several of the automated instruments may experience partial failure and/or may require maintenance that requires opening of the containment cabinet. In some cases, this may be performed by a medical laboratory scientist or by a company representative. We require that the operator wear face protection when performing any service functions.