The Journal of Infectious Diseases
SUPPLEMENT ARTICLE

Laboratory Response to 2014 Ebola Virus Outbreak in Mali

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Aware of the rapid spread of Ebola virus (EBOV) during the current West African epidemic, Mali took several proactive steps to rapidly identify cases within its borders. Under the Mali International Center for Excellence in Research program, a collaboration between the National Institute of Allergy and Infectious Diseases and the Malian Ministry of Higher Education and Scientific Research established a national EBOV diagnostic site at the University of Sciences, Techniques and Technologies of Bamako in the SEREFO Laboratory. Two separate introductions of EBOV occurred in Mali from neighboring Guinea, but both chains of transmission were quickly halted, and Mali was declared "Ebola free" on 18 January 2015 and has remained so since. The SEREFO Laboratory was instrumental in the success of Mali's Ebola response by providing timely and accurate diagnostics. As of today, the SEREFO Laboratory has tested 103 samples from 88 suspected cases, 10 of which were EBOV positive, since the Ebola diagnostics unit started in April 2014. The establishment of Ebola diagnostics in the SEREFO Laboratory, safety precautions, and diagnostics are described.

Keywords. Ebola virus; West Africa; epidemic; Mali; diagnostics.

Ebola virus (EBOV) emerged in West Africa, Guinea, in December 2013. Owing to high population mobility, porous borders, and poor public health infrastructure in this West African region, it developed into the largest reported EBOV epidemic. Guinea, Sierra Leone, and Liberia were overwhelmingly affected by the epidemic, experiencing >28 000 infections and >11 000 deaths [1].

Many West African countries activated their public health response systems and prepared a contingency plan for the emergence of EBOV in their countries. Surprisingly, only 3 additional countries encountered EBOV introductions during this epidemic. The most critical introduction of EBOV, in this case from Liberia, occurred in Nigeria in July 2014 and led to a total of 20 cases with 8 fatalities. It is interesting to note that the most densely populated country in the region, with about 175 million persons, managed to contain EBOV within 3 months [2]. In August 2014, Senegal reported the introduction of a single surviving case from Guinea with no further transmission [3]. Mali reported 2 independent introductions, both from Guinea in October and November 2014, with a total of 10 EBOV cases and 6 deaths [4].

Quickly confirming an EBOV diagnosis is important, not only for case patient management but also for limiting human-to-human transmission through isolation of infectious patients. For West Africa in general, samples from patients suspected of EBOV infection are sent to one of the World Health Organization (WHO) Collaborating Centers on Viral Hemorrhagic Fever in Africa (ie, Institute Pasteur, Dakar, Senegal; or National Institute for Communicable Diseases, Sandringham, South Africa), Europe (ie, Institute of Tropical Medicine, Antwerp, Belgium; Jean Mérieux BSL-4 Laboratory, Lyon, France; or Bernhard-Nocht-Institut, Hamburg, Germany), or the United States (Centers for Disease Control and Prevention [CDC], Atlanta, Georgia). Export and import regulations for such infectious specimens as well as shipment-related issues often delayed diagnosis by days or weeks. Therefore, to respond quickly to any potential spread of EBOV from neighboring Guinea, Mali assumed a proactive approach in spring 2014 by building the capacity to diagnose, isolate, and manage suspected EBOV cases. The first suspected EBOV samples arrived in April 2014, and testing has remained continual throughout the epidemic and thereafter.

MATERIALS AND METHODS

Biocontainment Facility

The biocontainment laboratory space (biosafety level [BSL] 3) was a room of approximately 4 × 6 m [24 m^2]) that was accessed through an anteroom (2 × 4 m; [8 m^2]) (Figure 1*A*). The BSL-3 space encompassed two 6-foot class IIA biosafety cabinets (6-foot; 1.8 m), 4 tissue culture/bacterial incubators, a −80C freezer, a refrigerator and a tabletop centrifuge. The room was

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The Journal of Infectious Diseases® 2016;214(S3):S164–8

DOI: 10.1093/infdis/jiw200

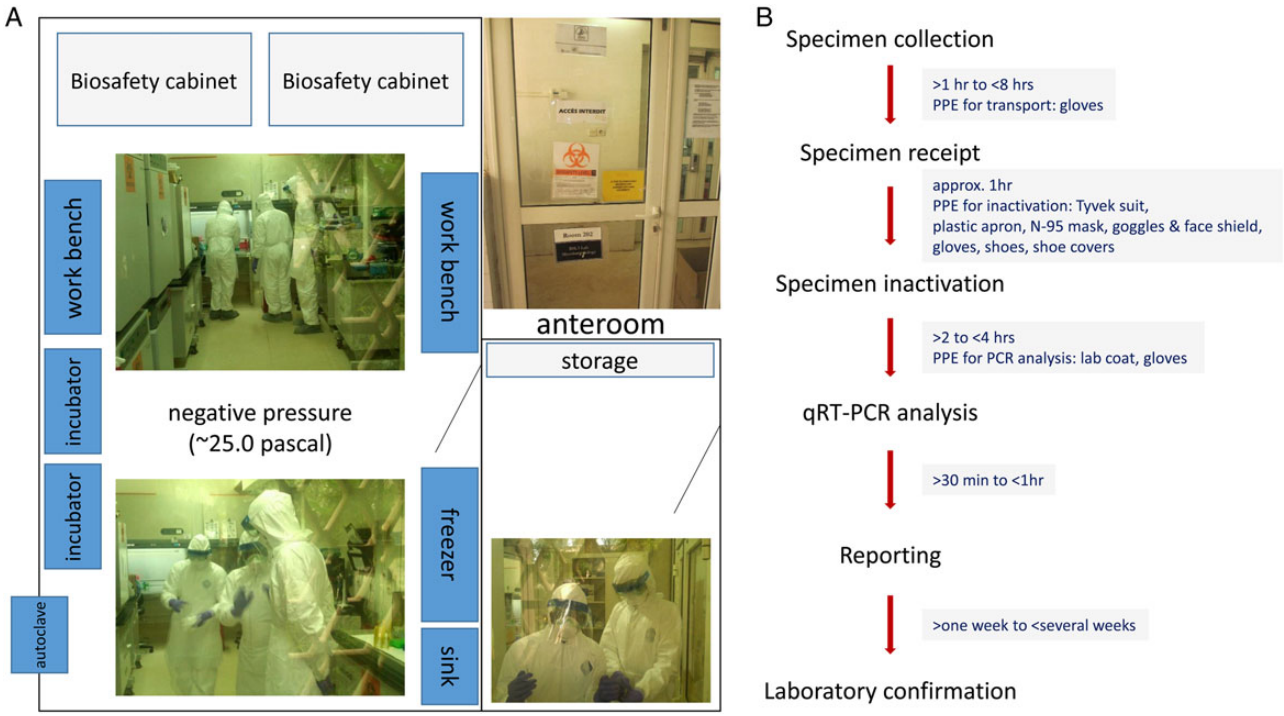

Figure 1. Biocontainment operation at SEREFO. *A*, Laboratory outline. Diagram represents the floor plan of the enhanced biosafety level (BSL) 3 containment space and the anteroom with key equipment and staff in personal protective equipment (PPE). The photo on the top-right offers a view into the anteroom with the entrance door to the BSL-3 space in the back. *B*, Workflow. Times are approximate. Laboratory confirmation was requested initially for all specimens and later on only for those positive for Ebola virus. Abbreviations: PCR, polymerase chain reaction; qRT-PCR, quantitative reverse-transcription PCR.

served by a pass-through autoclave for waste removal; all waste was first chemically inactivated and then incinerated on campus after sterilization. The room was operated under constant negative pressure to the anteroom (approximately −25.0 Pa). Proper performance of the BSL-3 space and the anteroom was checked every morning and before every entry.

Biosecurity

The laboratory was located inside the SEREFO Laboratory building, a component of the University of Sciences, Techniques and Technologies of Bamako (USTTB). Access to the SEREFO Laboratory was controlled by a card reader device, and a security guard was posted outside next to the entrance. The BSL-3 laboratory itself was accessed through the anteroom; a fingerprint device controlled access to the BSL-3 space (Figure 1*A*). Infectious specimens were kept in the freezer of the laboratory for short-term storage. Infectious clinical specimens were either removed from the laboratory for shipment to the CDC in Atlanta or destroyed by chemical inactivation followed by autoclaving and subsequent incineration.

Personal Protective Equipment

Staff changed into scrubs before entering the anteroom. Personal protective equipment (PPE) for entering the laboratory space included a Tyvek suit, plastic apron, N-95 mask, goggles and face shield, 2 pairs of gloves with the inner one taped to the Tyvek suit, and dedicated shoes and shoe covers (Figure 1*A* and 1*B*). Staff were trained on appropriate methods for doffing PPE after exiting the laboratory, including disinfection and the order in which to remove apparel.

Clinical Specimens

Whole blood was drawn into an ethylenediaminetetraacetic acid Vacutainer tube (3 mL). Oral, nasal, or skin swab samples were obtained with sterile cotton tips prewetted in viral transport medium. The cotton part of the swab was cut off and put into a 2-mL cryopreservation vial that sealed the specimen fluid. All clinical specimens were collected by trained medical personnel at the treatment and quarantine units.

Sample Receiving

Clinical specimens were delivered to the SEREFO Laboratory by dedicated vehicles adhering to national and international regulations (Figure 1*B*). The timeline for delivery was dependent on the location of the clinical ward (ie, border crossings to Guinea), but delivery usually occurred on the same day (within hours). For the treatment unit in Bamako, delivery occurred immediately (approximately 1 hour or ≤1 hour). Clinical specimens were delivered in double zipper storage bags in a type 1A container placed in a cool box. The accompanying paperwork was removed, and the samples were logged into a laboratory data sheet. The cool box was brought into the anteroom, and the

type 1A container was removed and carried into the laboratory (Figure 1*A*). The type 1A containers and cool boxes were reused after bleach disinfection (0.5%–1.0%).

Nucleic Acid Extraction

RNA was isolated from whole-blood or swab samples using the QIAmp viral RNA Mini Kit (Qiagen). Within a biosafety cabinet in the BSL-3 laboratory, individual clinical specimens (140 µL) were added to a tube with AVL Buffer (560 µL; prealiquoted), mixed, and left at ambient temperature for a minimum contact time of 10 minutes. The content was then transferred into a tube with 95%–100% ethanol (560 µL; prealiquoted), mixed, and left at ambient temperature for a 10-minute contact time. Tubes were spray disinfected and removed from the cabinet. On a clean bench, the inactivated material was again transferred into a clean tube, placed in a dunk tank containing 0.5%–1.0% bleach (prepared daily), and totally submerged for a minimum contact time of 10 minutes. The dunk tank was located in the anteroom in close proximity to the laboratory entrance door. Reliable and safe inactivation using AVL Buffer in combination with the subsequent ethanol addition step of the QIAamp Viral RNA Mini Kit protocol was evaluated before the field mission [5]. The remaining steps of the extraction protocol were performed according to the manufacturer's specifications within a class IIA biosafety cabinet in a designated BSL-2 laboratory with an additional wash step with AW1 buffer.

Quantitative Real-Time Polymerase Chain Reaction Assays

RNA was amplified with real-time quantitative reverse-transcription polymerase chain reaction (qRT-PCR) (Lightcycler 480 RNA Master Hydrolysis Probes; Roche) to detect EBOV using polymerase (L) gene-specific primers (5′-CAGCCAGCAATTTCTTCCAT-3′, 5′-TTTCGGTTGCTGTTTCTGTG-3′, and 2 probes: 56-FAM/ATCATTGGC/ZEN/RTACTGGAGGAGCAG/3IABkFQ and 56-FAM/TCATTGGCG/ZEN/TACTGGAGGAGCAGG/3IABkFQ). A qRT-PCR assay using EBOV nucleoprotein gene-specific primers (5′- TGCCGACGACGAGACGT -3′, 5′- CGTCCCTGTCCTGTTCTTCATC -3′ and /56-FAM/AGYCTTCCG/ZEN/CCCTTGGAGTCAGA/3IABkFQ) was designed as a confirmatory assay.

Control RNA from 3 ebolavirus species (*Zaire*, *Tai Forest,* and *Bundibugyo ebolavirus*) were included in the analysis. Each sample was also analyzed using a qRT-PCR specific for the detection of β_2-microglobulin (Applied Biosystems) to verify proper sample extraction and assay conditions. Thermocycling was performed on a SmartCyler instrument (Cepheid) using validated conditions. The detection limit of the L and nucleoprotein assays was 0.08 focus-forming units per milliliter of EBOV-Makona. The assays were evaluated through worldwide "Ebola proficiency panels 2014/2015" for RT-PCR diagnostics, produced at the Robert Koch Institute, Berlin, Germany, in close collaboration with WHO and other institutions.

Reporting of Diagnostic Results

Test results were released simultaneously to Médecins Sans Frontières personnel operating the Ebola treatment unit and the coordinator of the Operational Center for Emergency Response, who reported to the Ministry of Health. Reporting typically occurred within 4–6 hours after sample receipt but definitely on the same day (Figure 1*B*).

Ethics Statement

The clinical specimens included in this study were collected as public health surveillance and not as human subject research. Thus, submission to institutional review boards was not required.

RESULTS AND DISCUSSION

In spring 2014, about 6 months before the introduction of the first EBOV case into Mali, staff of the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), travelled to Bamako on official request by the Ministry of Health, Mali, to discuss the implementation of EBOV diagnostics. The decision to implement in-country diagnostics was due in part to a cluster of unexplained disease near the border of Guinea, which raised concern of the possible incursion of EBOV into Mali [6]. The SEREFO Laboratory within USTTB was selected as the laboratory site because it contained a BSL-3 facility operated by staff that was trained in handling virulent pathogens (Figure 1*A*). The SEREFO Laboratory was established in 2003 in collaboration between NIAID and USTTB, originally to develop tuberculosis and human immunodeficiency virus research programs.

As a first step, the existing BSL-3 laboratory operating procedures and protocols had to be adapted to an enhanced BSL-3 environment. For this, biosecurity was increased by activating existing card readers controlling and limiting access to the facility. Standard operating procedures/protocols (SOPs) were rewritten to reflect the increased level of biocontainment including enhanced PPE (Figure 1*A* and 1*B*). Selected SEREFO Laboratory staff began expanded training with NIAID scientists on those SOPs, the proper use of PPE and diagnostic assays for

Table 1. Laboratory Testing Since Spring 2014

	Year			
	2014	2015	2016	Total
Samples, No.				
Total	88	15	0	103
EBOV positive	10	0	0	10
EBOV negative	78	15	0	93
Equivocal results	0	0	0	0
Cases, No.				
Suspected	73	15	0	88
Laboratory confirmed	10	0	0	10

Abbreviation: EBOV, Ebola virus.

the detection of EBOV using real-time PCR. A dedicated BSL-2 area was selected and SEREFO laboratory staff were also trained on downstream noninfectious SOPs, such as RNA extraction, RT-PCR, and data interpretation.

As of March 2016, the SEREFO Laboratory has received and tested 103 samples from 88 suspected cases, 10 of which were EBOV positive (Table 1). The first positive sample was received and tested on 23 October 2014. The patient was a young child who had been traveling from Kissidougou, Guinea, into Mali by public transportation [7]. Owing to the rapid confirmation of the young case patient, 108 contacts were identified and monitored for disease for 21 days [8]. Fortunately, despite the child's traveling for hundreds of kilometers in a symptomatic state on a crowded bus, resulting in high exposure of other bus occupants and additional individuals, there was no human-to-human transmission [4, 9].

A second, separate introduction from Kouremale-Guinee, Guinea occurred shortly thereafter in November 2014 [4, 10]. An imam from Guinea travelled to Mali and subsequently developed symptoms consistent with EBOV infection. Unfortunately, samples were not collected, and the patient died while receiving medical attention in the capital city of Bamako. A nurse and a physician who had treated the patient in a private clinic tested positive in November 2014, which led to the identification of an additional 325 contacts, all of whom were followed up for 21 days to monitor them for EBOV symptoms. The importance of establishing EBOV diagnostics at the SEREFO Laboratory was illustrated when 5 of the 325 contacts tested positive for EBOV infection. The rapid identification and subsequent isolation of hundreds of primary contacts resulted in the quarantine of the 5 infected contacts and stopped this chain of EBOV transmission before it could spread throughout the population [11, 12].

All Ebola-positive specimens were sent to the CDC in Atlanta, which confirmed all positive samples, assuring the quality of EBOV diagnostics at the SEREFO Laboratory in Mali (Figure 1*B*). In addition, all EBOV-positive samples were also shipped to the NIAID Rocky Mountain Laboratories for sequence confirmation and genetic and phylogenetic analysis. The analysis of Mali EBOV sequences demonstrated a relative constant overall mutation rate of EBOV, comparable to findings in previous outbreaks [4], data that have been confirmed by others throughout the course of the epidemic [13–15].

During the EBOV outbreak, laboratory diagnostics was advanced with mobile laboratory capacity for deployment into remote areas should this have become necessary. The mobile unit included a glove box for safe sample inactivation and the SmartCycler system as the PCR amplifying platform, similar to what was implemented in previous filovirus outbreaks [16–18]. Because the unit has not yet been deployed in Mali, we refer to a more detailed description of a similar unit in another article in this supplement [19], a unit used in connection with a mobile laboratory deployment to Monrovia, Liberia, in 2014.

It is interesting to note that the introduction of EBOV into West African countries other than Guinea, Sierra Leone, and Liberia was rare and contained relatively quickly. Existing infrastructure, well-trained personnel, and activation of a response plan before EBOV introduction are likely key factors for this success. Senegal hosts one of a few WHO Collaborating Centers for Viral Hemorrhagic Fever (Institut Pasteur, Dakar, Senegal), and Nigeria has existing laboratory capacity established originally for Lassa Fever (Nigerian Center for Disease Control with diagnostic support from Lagos Teaching Hospital). The proactive approach of Mali to establish EBOV diagnostics probably was a significant factor contributing to the rather rapid containment of EBOV in Mali. However, other factors, such as increased awareness and surveillance at border crossings and hospitals and education of medical staff and the public, are certainly important contributors.

In January 2015, Mali was declared "EBOV free" and has remained in that status since then [20]. The SEREFO Laboratory continues to test suspected EBOV specimens as necessary, and to date it analyzed 11 suspected EBOV cases in this post–Mali outbreak period. In late 2014 and early 2015, the SEREFO Laboratory participated in a worldwide "Ebola proficiency panel" for RT-PCR diagnostics, with overall good performance but a few minor deficiencies (unpublished results). As a consequence, NIAID staff has performed repeat training sessions for SEREFO Laboratory staff on BSL-3 operation and continued improvement in EBOV PCR diagnostics.

In conclusion, in-country laboratory diagnostics are an important response activity to epidemics/outbreaks of infectious diseases, such as Ebola hemorrhagic fever. Rapid and reliable test results support case patient management and surveillance activities. Implementing diagnostic capacity in African countries will help the preparedness of the public health systems for future outbreaks and epidemics. Funding is critical and must be secured to maintain and build on existing capacity and train local personnel for future independent laboratory response.

Notes

Acknowledgments. We thank Kay Menk, William L. Shupert, and Julie Callison (all National Institute of Allergy and Infectious Diseases [NIAID], National Institutes of Health [NIH], Hamilton, Montana), as well as Mame Niang and Richard Sakai (both NIAID, NIH, Bamako, Mali), for logistical arrangements. We are grateful to Nafomon Sogoba, Ousmane Maiga, and Samba Diop (all University of Sciences, Techniques and Technologies of Bamako [USTTB], Mali) for technical assistance. We also thank Mark Pineda, Joseph Shott (both NIAID, NIH, Bethesda, Maryland), Robert L. Murphy (Northwestern University, Chicago, Illinois), and H. Clifford Lane (Division of Clinical Research, NIAID, NIH, Bethesda) for their help and support in Mali. Particularly, we acknowledge Ute Stroeher and Stuart Nichol (Viral Special Pathogens Branch, Centers for Disease Control and Prevention, Atlanta) for their diagnostic support and Allen Grolla, Jim Strong, and Gary Kobinger (Public Health Agency of Canada, Winnipeg), as well as Vincent Munster (NIAID, NIH), for providing oligonucleotide sequences. We also acknowledge the support by the Mali International Centers for Excellence in Research Program of the Division of Intramural Research, NIAID,

NIH. Finally, we thank the Ministry of Health and Public Hygiene and the leadership of the USTTB for their support.

A promising young Malian scientist, Sady Tounkara, lost his life before the publication of this article. We wish to honor Sady's memory.

Disclaimer. The funding sources of this study had no role in design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

Financial support. The work was supported by the Intramural Research Program of the NIAID, NIH, through the Mali International Center of Excellence in Research Program.

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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