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SUPPLEMENT ARTICLE

Ebola Laboratory Response at the Eternal Love Winning Africa Campus, Monrovia, Liberia, 2014–2015

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West Africa experienced the first epidemic of Ebola virus infection, with by far the greatest number of cases in Guinea, Sierra Leone, and Liberia. The unprecedented epidemic triggered an unparalleled response, including the deployment of multiple Ebola treatment units and mobile/field diagnostic laboratories. The National Institute of Allergy and Infectious Diseases and the Centers for Disease Control and Prevention deployed a joint laboratory to Monrovia, Liberia, in August 2014 to support the newly founded Ebola treatment unit at the Eternal Love Winning Africa (ELWA) campus. The laboratory operated initially out of a tent structure but quickly moved into a fixed-wall building owing to severe weather conditions, the need for increased security, and the high sample volume. Until May 2015, when the laboratory closed, the site handled close to 6000 clinical specimens for Ebola virus diagnosis and supported the medical staff in case patient management. Laboratory operation and safety, as well as Ebola virus diagnostic assays, are described and discussed; in addition, lessons learned for future deployments are reviewed.

Keywords. Ebola virus; West Africa; epidemic; mobile laboratory; diagnostics.

Ebola virus (EBOV) causes occasional outbreaks of viral hemorrhagic fever, termed Ebola hemorrhagic fever or, more recently, Ebola virus disease (EVD), in Central African countries such as the Democratic Republic of the Congo, the Republic of the Congo, and Gabon, with limited case numbers but high case-fatality rates of up to 90% [1]. Other ebolaviruses (Sudan virus and Bundibugyo virus) have been responsible for viral hemorrhagic fever outbreaks of similar dimensions in East African countries, such as the Republic of South Sudan and Uganda, but also northeastern Democratic Republic of the Congo, and there has been a single case of Tai Forest virus infection in Cote d'Ivoire. In addition, viral hemorrhagic fever outbreaks and episodes caused by marburgviruses have been reported from Kenya, Zimbabwe and South Africa, and Angola [1, 2]. Recently, the world witnessed the first EBOV epidemic in West Africa, starting in December 2013 in Guinea [3]. The 3 countries mainly affected were Guinea, Sierra Leone, and Liberia, with >28 000 EBOV cases and >11 000 deaths [4, 5]. All 3 countries have been declared EBOV free at least once since the epidemic began; unfortunately, all 3 countries have experienced relapses with temporary low-level EBOV transmission from mid-2015 to the time of writing. Virus persistence in or reactivation from immunologically privileged body sites leading to occasional human-to-human transmission, including sexual transmission [6, 7], has been discussed as a potential reason for the continued public health threat in the affected region during the postepidemic phase, yet it remains unclear to what extent, if at all, such factors may contribute to EBOV epidemiology [8, 9].

The unprecedented epidemic triggered unparalleled response activities by multiple government, academic, and private agencies/institutions worldwide. The response included the establishment of large numbers of Ebola treatment units (ETUs) and the deployment of many mobile/field diagnostic laboratories to different sites in all 3 affected countries [4, 5]. During the peak of the epidemic in Liberia, the National Institute of Allergy and Infectious Diseases combined forces with the Centers for Disease Control and Prevention (CDC) and deployed a joint laboratory to Monrovia (the CDC/National Institutes of Health [NIH] laboratory). The original plan was a deployment to Foya in the north of the country. However, because of rapidly increasing Ebola hemorrhagic fever case numbers in Monrovia, the capital and urban center of Liberia, a decision was made

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to set up the laboratory instead at the campus of Eternal Love Wining Africa (ELWA), a facility founded by the Christian mission organization Serving in Mission in 1965 [10]. The mobile laboratory initially supported the operation of the already operating ELWA2 ETU and the newly founded ELWA3 ETU, established and operated by Médecins Sans Frontières, which turned out to be the largest tent-structured field ETU any medical or aid organization had ever built in response to a filovirus outbreak [11]. Later on, samples were also processed for other ETUs located in the Monrovia area. Of note, several laboratories located at various sites in Monrovia and the country operated by different agencies and institutions have assisted Liberia in response to the Ebola epidemic.

MATERIALS AND METHODS

Personal Protective Equipment

Staff handling inactivated material wore scrubs, dedicated footwear (rubber boots/shoes), and a single pair of gloves. Staff performing centrifugation steps of the RNA extraction protocol following inactivation wore a face shield in addition. The operator of the glove box wore scrubs, a liquid impervious gown, rubber boots, an N-95 mask, a face shield, and double gloves with the inner pair taped to the arms of the gown. The enhanced personal protective equipment was required by the need for sample removal from the transport containers, waste removal from the glove box, and in case of unexpected power failure, which would result in the loss of negative pressure inside the glove box. Staff entering the mobile biosafety level 3 (BSL3) laboratory wore scrubs, dedicated rubber boots/shoes, a Tyvek gown, double gloves with the inner pair taped to the arms of the Tyvek gown, and a powered air-purifying respirator unit. To exit the BSL3 laboratory, gown and rubber boots/shoes were decontaminated with bleach (0.5%–1.0%) inside the BSL3 facility at the door to the anteroom, followed by removal of the outer pair of gloves. Subsequently, the person stepped into a tub with bleach solution (0.5%–1.0%) in the anteroom. A colleague wearing the same personal protective equipment as the glove box operator

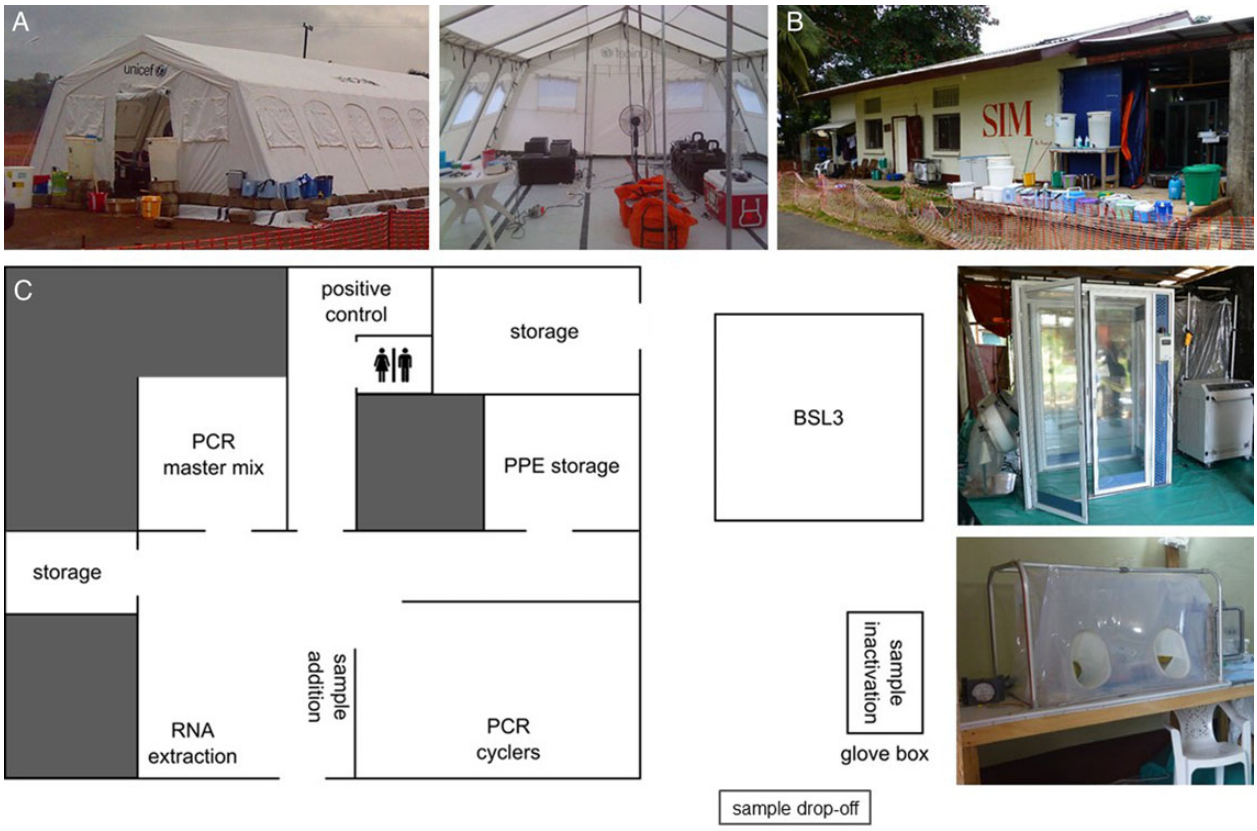

Figure 1. Laboratory set-up of the Centers for Disease Control and Prevention/National Institutes of Health laboratory at Eternal Love Winning Africa Ebola Treatment Unit 3, Monrovia, Liberia. The figure shows the initial tent-structure (*A*) and the fixed-structure (*B*) laboratory sites. The schematic (*C*) shows the floor plan of the fixed-structure building, separating the different areas for processing clinical specimens and for quantitative real-time polymerase chain reaction (PCR) diagnostic assays. The dark-gray areas were rooms not used for laboratory work. Only noninfectious material was handled in the building. Handling of infectious or potentially infectious clinical material was performed outside, at the sample drop-off area, in either the glove box (*C*, bottom photo) or the mobile biosafety level 3 (BSL3) laboratory (*C*, top photo). The glove box is shown with a view from the operator (bottom photo). The top photo shows the entrance to the anteroom of the mobile BSL3 laboratory, the waste removal device on the left-hand site, and the air handling system on the right-hand site.

performed a spray-disinfection with 70% alcohol in the anteroom before removal of the powered air-purifying respirator and remaining personal protective equipment.

Biocontainment Equipment

For inactivation of low sample volumes, we used a mobile glove box unit (Coy Lab Products, Grass Lake, Michigan; Figure 1). Negative pressure (20–40 Pa) was generated through a small vacuum pressure pump (Barnant Company, Barrington, Illinois), using intake and exhaust valves protected by double high-efficiency particulate air (HEPA) filters. The glove box has been field proven during previous laboratory support missions and has shown to be reliable and safe [12]. For the first time, we also used a mobile BSL3 laboratory, the IsoArk Portable Isolation Chamber (Beth-El Zikhron Yaaqov Industries, Israel; Figure 1).

RNA Extraction

RNA extraction was performed using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's specifications. We added an additional wash step with AW1 buffer to remove potential inhibitory substances of RNA amplification. The first 2 steps of the protocol—addition of AVL buffer and addition of 95%–100% ethanol—were performed in containment (ie, in the glove box or the mobile BSL3 laboratory). The remaining steps were performed outside of containment. Late during the outbreak, we tested automated RNA extraction using the MagMax Pathogen RNA/DNA kit and Dynal Bead Retriever (Applied BioSystems, Foster City, California). For each specimen, 100 µL of sample was added to 400 µL of lysis buffer for virus inactivation. Twenty-four samples in lysis buffer were processed according to the manufacturer's instructions during the 20-minute run.

EBOV Detection Assays

All clinical specimens were tested with quantitative real-time polymerase chain reaction (qRT-PCR) assays (Table 1). The L gene–specific qRT-PCR served as a primary detection assay throughout the mission and was designed specifically for the sequence of an EBOV-Makona isolate from Guinea [13] and used 2 probes. This assay was run on the SmartCycler (Cepheid, Sunnyvale, California) and the LightCycler 96 system (Roche, Indianapolis, Indiana) platforms. The NP gene–specific qRT-PCR was also designed specifically for the sequence of the same EBOV-Makona isolate from Guinea [13]; this assay was used for confirmatory testing throughout the mission and was run on the same platforms. The GP gene–specific qRT-PCR was also used as a primary assay, until November 2014. The GP gene primers were designed specifically for the species *Zaire ebolavirus*. This assay was run on the Joint Biological Agent Identification and Diagnostic (JBAID) system (Idaho Technology [now BioFire Diagnostics], Salt Lake City, Utah) platform. A VP40 gene–specific assay was run on the Bio-Rad platform (Bio-Rad, Hercules, California; primer and probe sequences are available upon request). As a housekeeping gene we used β-2-microglobulin with a B2M qRT-PCR (Applied Biosystems) applied to at least the EBOV-negative clinical samples to verify that each sample extraction and assay ran correctly. The detection limit of the L and NP 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, which were produced at the Robert Koch Institute (Berlin, Germany) in close collaboration with the World Health Organization (WHO) and other institutions. The CDC VP40 assay received Food and Drug Administration Emergency Use Authorization in March 2015.

Ethics Statement

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

Table 1. Comparison of Quantitative Real-Time Polymerase Chain Reaction Assays Used for Ebola Virus (EBOV) Diagnostics

Assay	Primer (Probe) Sequences	Platform(s)	Purpose	Comments
L gene specific	5′-CAGCCAGCAATTTCTTCCAT-3′, 5′-TTTCGGTTGCTGTTTCTGTG-3′ (56-FAM/ATCATTGGC/ZEN/RTACTGGAGGAGCAG/3IABkFQ; 56-FAM/TCATTGGCG/ZEN/TACTGGAGGAGCAGG/3IABkFQ)	SmartCycler and LightCycler	EBOV diagnostics; primary assay	Used as a primary assay from Aug 2014–May 2015 (no known performance issues)
VP40 gene specific	Available upon request	Bio-Rad	EBOV diagnostics; primary assay	Used as a primary assay from Dec 2014–May 2015 (no known performance issues)
GP gene specific	5′-AAGCATTTC CTAGCAATATGATGGT-3′, 5′- ATGTGGTGGGTTATAATAATCACTGACATG-3′ (56-FAM-5′CCAAAATCATC ACTIGTGTGGTGCCA-3)	JBAIDS	EBOV diagnostics; primary assay	Used as a primary assay from Aug 2014–Nov 2015 (no known performance issues)
NP gene specific	5′-TGCCGACGACGAGACGT-3′, 5′-CGTCCCTGTCCTGTTCTTCATC-3′ (56-FAM/AGYCTTCCG/ZEN/CCCTTGGAGTCAGA/3IABkFQ)	SmartCycler and LightCycler	EBOV diagnostics; back-up assay	Used as a back-up assay from Aug 2014–May 2015 (no known performance issues)
B2M gene specific	Commercial kit (Applied Biosystems, Foster City, California)	SmartCycler, Bio-Rad, and LightCycler	Extraction and amplification control	Used as a sample control assay from Aug 2014–May 2015 (no known performance issues)

Abbreviation: JBAID, Joint Biological Agent Identification and Diagnostic.

RESULTS

Laboratory Set Up

As a first laboratory site, a field across from the ELWA3 ETU was chosen that was a short distance from the ETU but outside the immediate security zone, thus avoiding patient contact. However, the location was within the ELWA campus security zone, enabling secure but independent operation while still providing instant contact with the ETU. The laboratory was established as a single-room tent structure (Figure 1*A*) with independent power support from a dedicated diesel generator and a smaller back-up generator. This laboratory site operated for approximately 1 month, from mid-August to mid-September 2014. Increasing sample volume, expansion of the ELWA3 ETU, and heavy rainfall during the rainy season led to the decision to move the laboratory to a fixed structure. The ELWA administration provided an unoccupied office building on the ELWA campus in close proximity to the ELWA3 ETU (Figure 1*B*). The interior layout of the building allowed separation of activities such as sample extraction, master mix preparation, sample addition, positive control addition, and platform analysis into different rooms or areas (Figure 1*C*). Only inactivated material was handled within the building. Receiving and handling of infectious material was performed outside the building. For this, a simple wooden roof structure was attached to one of the building's outside walls to cover and protect a space large enough to accommodate the mobile BSL3 laboratory and 2 glove boxes (Figure 1). The building and the outside area were normally powered by the ELWA campus generator, and a second diesel generator was set up on-site as a back-up during power outages. On-site logistical support for the laboratory and its operation was provided by trained Médecins Sans Frontières personnel from the ELWA3 ETU.

Operation of Biocontainment Equipment

The glove box was equipped with a pass-through chamber and closable intake and exhaust air valves and assembled per the manufacturer instructions. A negative-pressure environment was produced using a small vacuum pump; intake and exhaust air underwent single and double filtration, respectively, with a HEPA filter. Prior to operation, the glove box underwent safety testing for leaks and was set to maintain an operating negative pressure in the range of 20–40 Pa. The gloves on the glove box were changed once per week or more frequently, when needed. Upon starting the daily diagnostic routine, the glove box pressure was checked, the vacuum pump was turned on, and the valves for exhaust air (first) and intake air were opened to allow for the appropriate operating pressure to stabilize. The inner surface was wiped down with 70% ethanol and dried with paper towels. The waste from the previous day was sealed in double Ziploc bags with spray-based disinfection (with 0.5%–1.0% bleach) in between. The chlorine-disinfected waste was moved into the pass-through chamber, disinfected by spraying with bleach (0.5%–1.0%), for a minimum contact time of 10 minutes, removed from the chamber, and placed into a sealed plastic container for incineration. All required material and equipment for operation entered using a pass-through chamber on the right-hand side of the glove box. All infectious work was performed on absorbent disposable underpads (bench covers) that were changed daily or as needed, to avoid contamination of the glove box. After concluding work with infectious material, equipment such as pipettes underwent spray-based disinfection with 70% ethanol, were wiped down, and were placed into large Ziploc bags to avoid corrosion due to bleach fumes. Subsequently, the inner surface of the glove box was disinfected by spraying with 0.5%–1.0% bleach; the use of bleach for disinfection was minimized as time passed as this affected the transparency and lifetime of the glove box material. Finally, the intake and exhaust air valves were closed, and the glove box was powered down over night, maintaining a negative pressure of >25 Pa.

The mobile BSL3 was set up as a single operating space that was entered and exited through an anteroom (Figure 1). An air conditioner unit was installed to control temperature and humidity during operation; a work bench was installed for handling of samples and all associated procedures. Prior to operation, the BSL3 laboratory was visually inspected for damage, and the unit was set to maintain an operating pressure in accordance with the manufacturer's instructions. Materials and equipment were carried in by the operator through the anteroom. After finishing work with infectious material, equipment such as pipettes and bench-top spaces underwent spray-based disinfection with 70% ethanol and were wiped down. Disinfected waste was removed through an incorporated pass-through chamber, allowing biohazard bags to be sealed (Figure 1*C*; left side of the anteroom). The bags were disinfected by spraying with 0.5%–1.0% bleach and placed into a sealed plastic container for transport to the incinerator.

All disinfected waste from the glove box and mobile BSL3 laboratory was placed into biohazard waste bags, sealed in larger plastic containers, and transported to the ELWA3 ETU for incineration on-site. Incineration was normally started within a few hours after receipt of waste but invariably on the same day.

Sample Collection, Transport, and Receiving

Blood samples were collected by clinical personnel in the ETU, using 3-mL ethylenediaminetetraacetic acid–containing Vacutainer tubes. Oral, nasal, or skin swab specimens were collected with sterile cotton-tipped plastic swabs and placed into 1 mL of viral transport medium in a 2-mL cryopreservation vial by breaking off the cotton tip to allow for the lid to seal the tube. The sample tubes were labeled with an indelible marker and provided information on patient identifiers and names (first and last name). Blood collection tubes and sealed cryopreservation vials underwent spray-based disinfection from the outside with bleach, placed and sealed in double Ziploc

bags that were also disinfected by spraying with 0.5%–1.0% bleach, and put in either a closed household bucket or handheld cooler, which were again disinfected by spraying the outside with 0.5%–1.0% bleach prior to transport. ETU personnel delivered samples directly to the laboratory site. Later, samples were also received from Global Communities and Riders for Health personnel, who collected samples from hospitals in town and delivered them to the laboratory. Patient data sheets that listed patient information, including a patient identifier number, name, sex, age, home address, and onset of clinical symptoms, accompanied each clinical specimen. After receipt of the samples at the laboratory site, samples were assigned a laboratory identifier and were logged into a laboratory spreadsheet. The spreadsheet was shared with the headquarters of the Ebola Incident Management System [14], the ETU, or other admitting entities, and a list of identified organizations/individuals every evening or upon request during the day.

Sample Inactivation and RNA Extraction

Sample inactivation was mainly performed in the glove box but, in some instances, also in the mobile BSL3 laboratory. 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 prior to the field mission [15]. For sample inactivation, 140 µL of clinical sample was added to 560 µL of AVL buffer in prealiquoted tubes (red lids were used for identification) followed by vigorous shaking and a contact time of 10 minutes at ambient temperature. The AVL/sample mixture was then removed from the tube and added to 560 µL of 95%–100% ethanol prealiquoted in a new tube (yellow lids were used for identification), followed by vigorous shaking at ambient temperature. After spray-based disinfection (0.5%–1.0% bleach) of the gloves of the operator, the tubes with the yellow lids were moved into a dunk tank containing fresh (prepared daily) 0.5%–1.0% bleach and placed in the pass-through chamber (glove box) or anteroom (mobile BSL3 laboratory). The tubes were then totally submerged for a minimum contact time of 10 minutes at room temperature. The tubes were removed and transported to the noninfectious work area for RNA extraction. The efficiency of extraction by using this procedure was previously evaluated and compared for selected clinical specimens [16]. Late during the outbreak, we tested automated RNA extraction using the MagMax Pathogen RNA/DNA kit and Dynal Bead Retriever. However, this system was not implemented during the CDC/NIH laboratory operation because of decreasing sample volume at the time. Automated extraction should be considered for future outbreak support missions of similar scale because sample processing efficiency and, likely, quality can be increased.

EBOV Diagnostic Procedures

The number of laboratory personnel was adjusted to the workload over the course of the mission. During the peak of the epidemic in Monrovia, the team consisted of 5 international members; this was later reduced to 4 and, finally, to 2. The laboratory operation was supported by a local staff member who helped with the day-to-day logistics and cleaning of the noninfectious laboratory space. In addition, the teams were provided with a driver from the US embassy for their entire stay.

Samples were received throughout the day, but sample processing was performed in batches in the morning (9:00–10:00 AM), early afternoon (1:00–2:00 PM), and late afternoon (4:00–5:00 PM) to allow a more efficient laboratory operation. If specimens were considered urgent by the ETU, usually so-called emergency specimens, they were processed individually at any time of the day but avoiding night for security reasons and for lack of consequences for patient management, as there was in general no discharge or patient movement within the ETU during the night. The normal turnaround for test results was approximately 4 hours from sample receipt, depending on the number of samples processed simultaneously. Urgent requests were processed and results reported in about 2.5 hours (sample inactivation, RNA extraction, and sample analyses took approximately 45 minutes, 45 minutes, and 60 minutes, respectively).

Each specimen was tested in at least 2 independent qRT-PCR assays targeting distinct regions of the EBOV genome. In case of discrepancy in test results, a third independent confirmatory assay was performed. From August until November 2014, we performed, as our primary assays, an L gene–specific and a GP gene–specific qRT-PCR on the SmartCycler and JBAIDS platforms, respectively. In November 2014, we replaced one of the primary assays, the GP gene–specific qRT-PCR, with a VP40 gene–specific qRT-PCR that was performed on the BIORAD CFX96 platform. The L gene–specific and VP40 gene–specific qRT-PCRs were performed as the primary assays until closure of the laboratory, in May 2015. A NP gene–specific qRT-PCR served as the confirmatory test throughout the mission and was performed on the SmartCycler platform. Owing to increased sample volume, the initial SmartCycler platform was replaced by the Roche LightCycler 96 platform in November 2014; this was possible as the NP gene–specific and L gene–specific assays were designed to be compatible with both platforms. For at least all EBOV-negative specimens, we performed an independent qRT-PCR for a housekeeping gene (B2M) as an internal sample and extraction control. In case of a B2M-negative result, the sample was reextracted or a new sample was requested.

Laboratory testing and reporting was based on an established algorithm. If a sample tested positive by 2 assays ($Ct < 35$; later, $Ct < 37$), the sample was reported EBOV positive, with the Ct value for the L gene–specific assay reported. In cases where the result of 1 test was positive and the result of the other was negative or equivocal, we ran a third independent confirmatory assay (NP gene target). In cases of positive results for the third assay, we would report EBOV positive but request a follow-up sample. EBOV-negative patients with continuing clinical

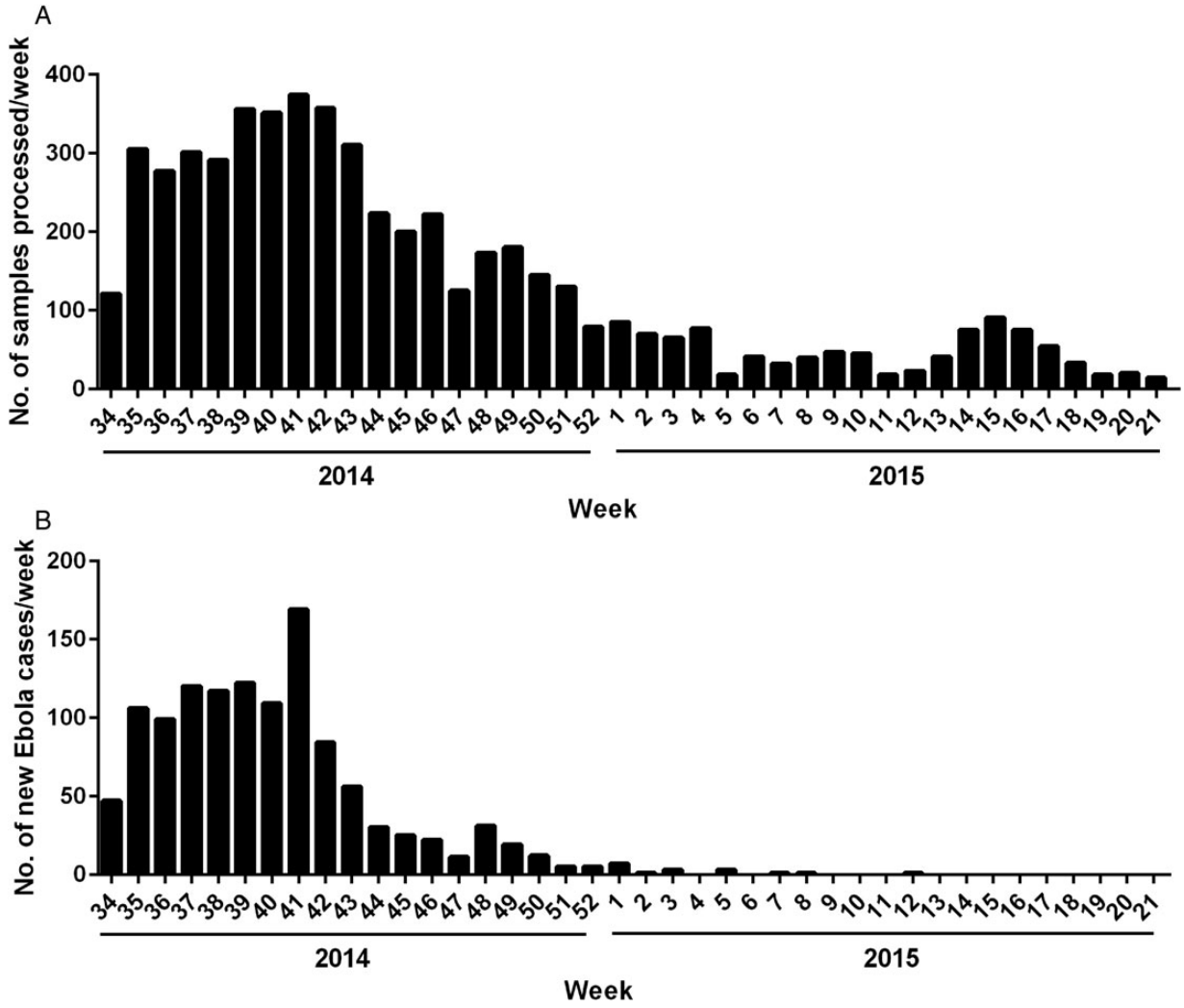

Figure 2. Sample load and Ebola virus (EBOV)-positive case numbers, by week of operation period. The top graph (*A*) shows the workload, and the bottom graph (*B*) shows the number of EBOV-positive specimens per week from August 2014 (laboratory start) until May 2015 (laboratory closure).

symptoms were re-tested after 24–48 hours (sometimes 72 hours), during which they were held in the so-called suspect ward. This triage procedure was lifted during the peak of the epidemic owing to lack of space in the ETU suspect ward. Patients with 2 consecutive EBOV-negative specimens (Ct > 35; later, Ct > 37) were usually discharged from the ETU suspect ward; at peak times of the epidemic, they were discharged after a single negative laboratory test result. Occasionally, >1 retest was requested, based on clinical evaluation and parameters. During the peak of the outbreak, retests of EBOV-positive cases were only done on survivors after 3 days without symptoms. Those patients were tested until a Ct of >35.0 was observed; in October 2014 this was changed to a Ct of >37.0. These cutoffs were chosen on the basis of experience from previous outbreaks and a general lack of the ability to isolate virus from specimens (humans or experimental animals) with these or lower viral loads [17] (our unpublished data).

The workload of the laboratory changed over time, and Figure 2 presents sample loads per week over the entire operating period from August 2014 until May 2015. From August until November 2014, sample numbers were almost always >200/week. Over the next 2 months, sample volume dropped to about half, and over the subsequent months until closure of the laboratory in May 2015, the sample volume dropped steadily, with the exception of April 2015, for which the volume was back to the level in January 2015. This increase was related to heightened contact tracing triggered by a potential sexually transmitted case around mid-March [7, 18], as well as routine blood samples collected by hospitals and ETUs in the area. Until mid-October 2014, >100 EBOV-positive cases were diagnosed per week. Subsequently, the number of EBOV-positive cases dropped steadily, and toward the end of the operation only sporadic positive cases were detected. For almost the entire period, sample inactivation could be handled in the glove box. However, the mobile BSL3

laboratory proved reliable and safe operation over 9 months and can be considered a realistic alternative for future outbreaks/epidemics if sample volume is expected to be high.

Additional Diagnostic Assays

In October 2014, the laboratory started to offer qRT-PCR for *Plasmodium* species. We used a previously published assay designed to detect all known human pathogenic species [19]. A description of the assay and an evaluation of the benefits of performing *Plasmodium* species parasitemia testing during EBOV outbreaks/epidemics were presented and discussed in a previous publication [20].

During the period from early December 2014 to May 2015, the laboratory also started to perform blood chemistry testing on selected serum samples from the ETU. We used the Piccolo Xpress chemistry analyzer (ABAXIS), a portable device that offers a full complement of Clinical Laboratory Improvement Amendments–waived blood chemistry tests. For safety reasons, the device was operated in a separate glove box. A description and evaluation of blood chemistry testing is presented and discussed in a different publication of this supplement of *The Journal of Infectious Diseases* [21].

DISCUSSION

Despite previous field laboratory experience among individual team members during past filovirus outbreaks, the dimension of this epidemic and the large volume of samples created a unique situation causing multiple logistical issues and concerns for laboratory operation and safety.

The weather conditions, particularly those during the rainy season, made operation out of a tent structure cumbersome. Heat, rain, humidity, and wind had an impact on machine operation and performance. In addition, the tent structure did not provide a sufficient level of security for equipment and supplies. The move to a fixed structure was beneficial and solved most of the weather- and security-related issues. The installment of a cooling unit in the building allowed for proper temperature and humidity control and more-reliable storage conditions for reagents and clinical specimens. The fixed structure also addressed most of the security issues, as proper locking systems could be implemented in addition to oversight by guards during the night.

Despite high sample volumes from August to November 2014, we were able to perform sample inactivation in the glove box system. This, however, led to some delays (usually not more than a few hours) in reporting, as sample throughput in the glove box was low. We set up the mobile BSL3 laboratory in September 2014 in preparation for a high sample volume. Although, we only used the mobile BSL3 laboratory sporadically for specimen inactivation procedures, the set-up performed well and was safely operated over the entire laboratory operation period, with no technical failures. This is encouraging and makes the mobile BSL3 laboratory a realistic alternative for potential future outbreaks/epidemics of a similar scale.

Safe and reliable sample inactivation is mandatory for all containment operations including mobile laboratories in the field. Those methods should be evaluated for proper inactivation prior to use. The RNA extraction kit used here did not completely inactivate EBOV after the addition of AVL buffer; reliable inactivation was only achieved after subsequent ethanol addition, and consequently this step was performed before samples were removed from containment [15]. RNA extraction procedures, and in particular commercial RNA extraction kits, should undergo safety testing for proper inactivation to determine the appropriate step in the protocol that would allow for safe removal from containment. Of note, this requirement does not question the quality of these extraction kits, as these kits are not designed specifically for containment work.

The high number of patients admitted to the ETU triage areas during the peak of the epidemic in Monrovia and the lack of available space in the so-called suspect and confirmed wards of the ETUs called for less stringent case patient management and corresponding adaptation of the laboratory algorithm. Normally, suspect cases would be tested upon arrival at the ETU triage area and, in the case of a negative result, would be retested in 24–72 hours. This did not always occur from August through November 2014. Likewise, convalescent survivors from the confirmed ward would normally be retested until a negative laboratory result was obtained, which took days or weeks in some cases. The situation in the confirmed ward at peak times of the epidemic did not allow for holding patients without clinical symptoms for a long period. For those cases, a cycle threshold cutoff of >35.0 (later, >37.0), in conjunction with clinical parameters, was established as a guideline for discharge. Of course, such guidelines presented a risk of discharging a patient during the EBOV incubation period or discharging a survivor, who might still be infectious even though the risk was minimal. However, this risk needed to be balanced with the risk of turning away suspect cases at the triage area owing to a lack of bed capacity in the wards. This situation changed with the drop in case numbers in late 2014. Therefore, it is recommended that the laboratory operate in close proximity to and in constant communication with the medical staff of the ETUs, allowing for frequent discussion on adaption of such algorithms in real time to achieve optimal service under the given circumstances.

Molecular diagnostic assays, in particular qRT-PCR, which was largely used during the recent EBOV epidemic, including at the Monrovia laboratory, are susceptible to failure due to genetic changes occurring during EBOV replication. During the West African epidemic, EBOV showed an overall substitution rate of approximately 1.3×10^{-3} substitutions/site/year, which is relatively consistent with reports from previous outbreaks [22–25]. Despite not mutating more frequently, mutations still occur in the EBOV genome as a result of viral replication. To our knowledge, however, there are no reports in the literature

for diagnostic assay failures during the West African epidemic due to such genetic changes. To avoid this issue, molecular diagnostic assays should be based on at least 2 genome targets; a third back-up target would provide further assurance, particularly in evaluation of clinical specimens with equivocal results. During smaller outbreaks, conformation may also be achieved by an independent test, such as antigen-detection enzyme-linked immunosorbent assay. This assay, however, appears to be less sensitive and less specific and could cause safety issues in the field [1]. Current platforms for molecular diagnostic analysis easily allow for multiplexing of assays, and multitarget diagnostic tests seem easy to achieve. In our operation, we achieved high concordance among 4 qRT-PCR assays targeting distinct regions in the EBOV genome, designed by independent institutions and run on multiple platforms, allowing for rapid, reliable and confirmed diagnosis in a field situation.

In conclusion, mobile laboratory diagnostic assays are a tremendous asset for the public health response to outbreaks/epidemics caused by EBOV or related pathogens. Early and proper mobilization is key in supporting case patient management, community surveillance, and convalescent follow-up. Safe and reliable operation of those laboratories is of the utmost importance, as well, as is a close collaboration with medical staff in the treatment units. As more diagnostic platforms become suitable for field use (ie, loop-mediated isothermal amplification and rapid antigen detection tests), laboratory set-up and operation needs to be adapted and optimized. The same holds true for safety equipment for sample inactivation. The description and evaluation of the Monrovia mobile laboratory, as presented here, together with descriptions of other mobile laboratory operations from previous outbreaks [12, 26] and the current West African epidemic [27, 28], may serve as examples and models to guide future outbreak missions.

Notes

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