

Ebola Virus Disease Outbreak, Sierra Leone – Laboratory Response Viral Hemorrhagic Fever Consortium

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1.0 Abstract

In response to the spread of Ebola Virus Disease (EVD) into Sierra Leone, long-standing research partners of the Kenema Government Hospital (KGH), in collaboration with the Sierra Leone Ministry of Health and Sanitation (MoHS), quickly mobilized to provide laboratory and diagnostic support to the hospital. The two partners involved in this effort are (1) the Viral Hemorrhagic Fever Consortium (VHFC) which includes partners from Tulane University, Harvard University, The Broad Institute, Scripps Institute, Redeemer's University, and Corgenix, and (2) Metabiota, which includes partners at Global Viral and USAMRIID. For brevity, we will refer to them as VHFC and Metabiota going forward. We also note that while VHFC support operations, we are not performing diagnostics; rather, we train and enable local teams to perform diagnostics. The two teams performing diagnostics are the MoHS Lassa fever (LF) laboratory at KGH (referred to as the KGH Laboratory in the following) and Metabiota. VHFC is treating outbreak response as the foremost concern, and has suspended all research activities on-site; we are providing all the resources we can make available to aid in the response.

The goal of this report is to describe current efforts by KGH (with support from VHFC) in coordination with Metabiota and to provide insights into existing laboratory diagnostics for EVD. We will outline the current operations at the KGH Laboratory, including diagnostic assays, sample processing, and disease reporting. We hope this report will aid in improvement of laboratory efforts, clinical care, and containment.

2.0 Background

KGH is one of only two permanent sites known to us in West Africa focusing on the diagnosis and treatment of Viral Hemorrhagic Fevers (VHFs); the other is Irrua Specialist Teaching Hospital in Nigeria. For ten years VHFC has worked closely with KGH to develop standard of care diagnostics for Lassa virus (LASV) and to provide support for the heavy disease burden (Shaffer et al., 2014.). The VHFC program is an extension of the now-concluded CDC *Guinea Lassa Fever Research Program* that Dr. Dan Bausch and others established for CDC's LF activities in West Africa from 1996-2002.

Through this partnership, KGH has become a regional referral center for LF patients. It is equipped with an LF ward, as well as a biocontainment laboratory capable of processing human samples and performing diagnosis in a field setting. The laboratory was established at KGH for diagnosis of LF and other viral diseases. Patient samples are manipulated in class II biosafety cabinets by personnel wearing full personal protective equipment (PPE) (gowns, gloves, and mask). The KGH Laboratory is located on the grounds of the hospital, but in a stand-alone building constructed in 2005. Testing is performed in a specialized containment suite for manipulation of samples from suspected cases of LF (Figure 1). ELISA (antigen capture, as well as IgM and IgG antibody) and PCR for LF are performed following standardized protocols. LASV antigens, monoclonal antibodies, and other reagents for ELISA are provided by VHFC.

Following the Guinean EVD outbreak detected in March 2014, VHFC established a field team comprising Dr. Kristian Andersen and Stephen Gire (Harvard/Broad) to provide supplies and technical support to KGH for combating the possible threat of EVD spreading to Sierra Leone.

At that time, they provided laboratory biosafety training and PPE to anticipate potential work with EVD patient samples. They also helped optimize PCR assays for Ebola virus (EBOV), based both on published sets of primers (Panning et al., 2007; Adrienne et al., 2010), and a new set of primers based on EBOV sequences closely related to the Guinean strains (Baize et al., 2014), for rapid field deployment and long-term surveillance. Simultaneously a team from Metabiota led by Dr. Nadia Wauquier initiated real-time PCR (qPCR) assays employing primers developed by the USAMRIID Program.

On May 25, 2014 Mr. Augustine Goba, Director of the KGH Laboratory used primers and protocols supplied by VHFC to identify the first EVD case in Sierra Leone by PCR (referred to as the KGH EBOV assay). A woman presented on that day to the KGH Maternity Ward with viral hemorrhagic fever (VHF) symptoms. The patient had also suffered a spontaneous abortion. Because of the importance of LF in pregnancy, there was a high suspicion that the patient was infected with LASV. A sample was sent for testing to the KGH Laboratory. However, the VHFC rapid test for LF was negative as were other recombinant LASV (reLASV) assays that test for the presence of LASV antigen or anti-LASV IgM or IgG.

Mr. Goba then tested the sample and determined that it was positive on the KGH EBOV assay. Dr. Wauquier of Metabiota confirmed the test, and a sample was also subsequently sent to Harvard University and the Broad Institute for further confirmation by sequencing. A dilation and curettage (D&C) procedure was performed on the patient. She survived and subsequently was the first EVD patient discharged from KGH. The maternity ward staff was carefully monitored for fever and other signs of EVD. No EVD cases occurred among KGH staff that attended this case.

In light of the EVD outbreak in Sierra Leone, VHFC team members have suspended research activities on-site, and have instead focused on providing whatever resources we have available to those responding to the outbreak. As described above, the partners from the VHFC have trained laboratory staff at KGH, including three technicians supported by VHFC, to perform PCR EBOV diagnostics. We continue to support clinical staff at the KGH, including full support for 12 nurses and partial support for one physician specialist. VHFC has provided supplies to increase bio-safety in the KGH laboratory, and has supported EVD case investigations, contact tracing, community education and sensitization. It is also providing logistical support, including the use of two land cruisers (along with fuel, per diems and hazard pay) for transport of blood samples of suspected EVD cases, as well as support for patient transport of EVD and LF cases to KGH and the burial of EVD victims. It continues to support administration and facilities staff at KGH (the program coordinator, one IT person, one facilities

manager and five cleaners). Total expenditures by Tulane for the period of March to mid July, including only direct funds to KGH, and supplies sent to KGH, are \$237,000, not including Tulane personnel. Harvard University and the Broad Institute have also supplied initial reagents and supplies to KGH, carried out training and enhancement trips, and performed sequencing. Total expenditures by Harvard/Broad thus far are \$116,919, not including Harvard/Broad personnel.

As the EVD outbreak continues to spread to new districts in Sierra Leone, and as the burden of disease increases, VHFC is compiling the following report to provide detailed information on laboratory preparedness, laboratory procedures, diagnostic assays, and disease reporting at the Kenema site in Sierra Leone. We also describe briefly other concurrent efforts. Finally, we provide background for your consideration on immediate and long-term priorities to continually enhance practices at the hospital.

3.0 Laboratory Preparedness

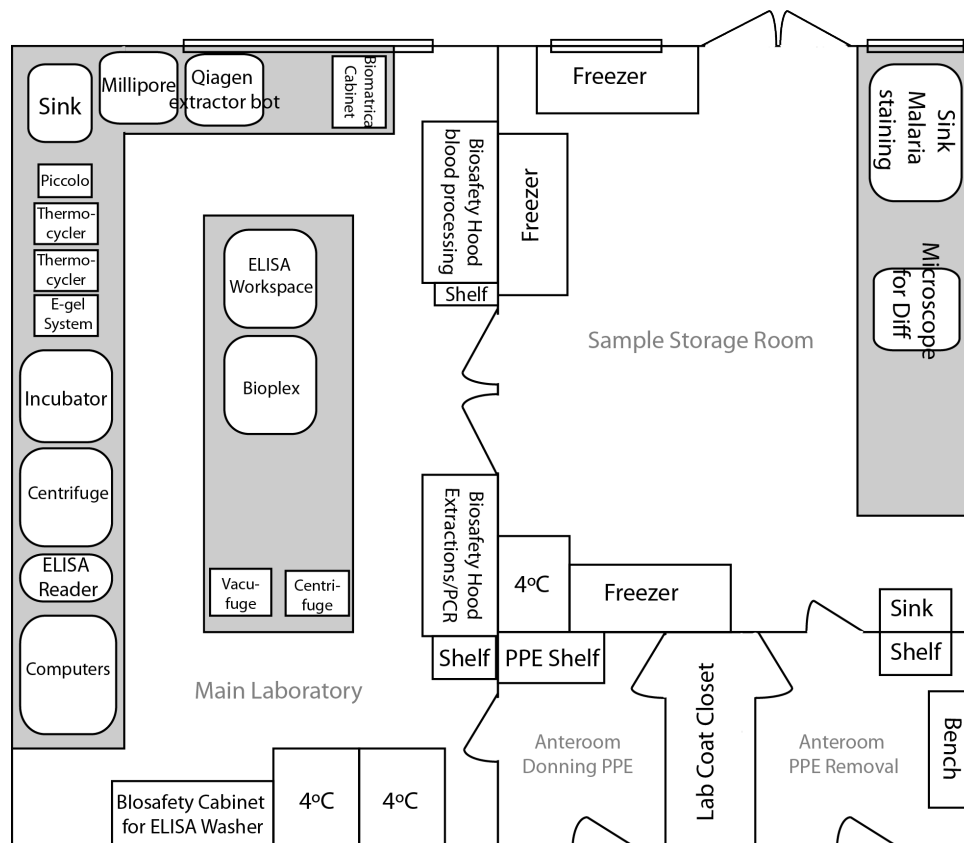
The KGH Laboratory is a 700 square-foot specialized suite for manipulation of samples from suspected cases of LF (Figure 1). The laboratory possesses equipment and trained personnel for diagnostics using traditional PCR and qPCR, ELISA, and immunofluorescent antibody tests. The building is equipped with redundant power sources, including town power in Kenema (which is extremely sporadic), 100 and 12 kilovolt amps (kVA) generators, and solar panels. Established biosafety and biosecurity guidelines are maintained and continuously monitored.

To strengthen the established biosafety guidelines at the KGH Laboratory, a team from VHFC was deployed in late March 2014, in order to anticipate the potential spread of EVD to Sierra Leone and to provide further training and biosafety guidelines for local staff to work safely with suspected and confirmed EVD cases. Specific improvements to biosafety in the KGH Laboratory are as follows.

1. Strict decontamination protocols were instituted for the main laboratory space both prior to work and after work completion at the end of the business day.
2. Comprehensive PPE guidelines were strengthened, including requirements for hooded coveralls, N95 masks, face shields, long-sleeved gloves, a splash resistant gown, foot covers, and an additional set of gloves in case of glove breakage.
3. Signs were also posted in laboratory spaces outlining safety guidelines for work with EVD, including all relevant procedures.
4. The Qiagen extractor robot was discontinued by Metabiota staff on all suspected or confirmed EVD cases to minimize aerosol.
5. An additional biosafety cabinet was placed in the main laboratory space in order to handle ELISA washing for the LF ELISAs. Metabiota has been using this for performing antigen-capture ELISAs on EVD positive samples. After the first Sierra

Leonean case was diagnosed, the KGH team performed a preliminary evaluation of several EBOV ELISA assays. KGH is not performing any EBOV ELISA assays.

A



B



C



Figure 1: The KGH laboratory. (A) Schematic of laboratory layout. A 700 square-foot specialized suite for manipulation of samples from suspected VHF cases. (B) A panoramic view of the inside of the diagnostic laboratory. Equipment not seen on the back wall are two additional biosafety hoods (there are currently 3 in the lab), as well as equipment in the sample storage room. (C) PPE used by staff while performing work in the laboratory.

In addition, the KGH Laboratory with support from VHFC implemented three separate traditional PCR-based EBOV assays. These assays were tested at KGH to determine which were most reliable and accurate for long-term implementation at the KGH Laboratory. The specific goals of assay implementation were to screen collected suspected cases of EVD, and to provide a long-term solution for current and future surveillance of EBOV in the region. For detailed information about the PCR assays used by KGH, please refer to section 6 on PCR diagnosis. These assays were responsible for detecting the first case of EVD in Sierra Leone on May 25th, 2014 (see above). Metabiota has concurrently implemented qPCR based diagnostics based on assays provided by USAMRIID.

4.0 Sample Processing

Starting in late March 2014, both KGH (with support from VHFC) and Metabiota began testing suspected EVD cases in the main suite at the KGH Laboratory. The two teams have a long-standing protocol put in place for LF patients, where both teams utilize whole blood from patients for diagnosis, clinical care monitoring, and approved research initiatives. This same protocol is maintained for all EVD patients at KGH. In brief, one 8mL EDTA tube of blood is collected from each patient. This blood is either collected in the hospital (for arriving new patients), in the EVD/LF ward (for EVD-confirmed patients) or at remote sites throughout the country (for individuals who are in remote regions and for whom only a blood sample is sent to the KGH Laboratory for diagnosis). A small amount of blood is removed from the EDTA tube and a manual differential is performed, as well as a basic metabolic panel assay (Piccolo, Abaxis) in the ELISA hood for additional safety. The blood tube is then split evenly between the two groups.

The KGH staff process the sample as follows:

1. Whole blood is spun down in a swinging-bucket centrifuge with bucket lids.
2. Serum is removed and aliquoted into 2-3 tubes containing AVL lysis buffer (Qiagen, Inc), which has been safety tested and determined to inactivate cell free media from EBOV cultures (USAMRIID). These aliquots are used for diagnostics on-site. One aliquot is also stored to be sent to Harvard University, where confirmatory qPCR diagnosis is performed on each sample using published primers (Adrienne et al. 2010) – provided by USAMRIID. This aliquot is also used for Next Generation sequencing, an effort endorsed by the MoHS as an emergency response effort to the EVD outbreak.
3. An aliquot of unadulterated serum is also removed for serology with LASV assays (only for samples that are not confirmed EBOV positive), as well as stored in case it might be helpful to international collaborators such as the CDC, WHO, NIH, USAMRIID, and other partners.

4. Buffy coat is isolated and inactivated in Trizol, which has been safety tested by USAMRIID for whole cell media/tissue, and then stored at -20°C. This sample is stored as clinical excess.

5.0 RNA Extractions

In order to extract viral nucleic acids from patient samples and perform RNA-based diagnosis (PCR and qPCR, referred to simply as PCR), serum or plasma from patient samples are inactivated in Qiagen AVL lysis buffer and incubated for 10 minutes at room temperature according to the manufacture's recommendations and approved inactivation protocols established by USAMRIID. Inactivated patient samples are then extracted using standard protocols for the Qiagen QIAamp Viral RNA Mini Kit. The extracted RNA is then used in designated PCR diagnostic assays for EBOV.

Initially both KGH and Metabiota were performing separate extractions in the one designated extraction hood and PCR in the main laboratory (Figure 1). While this provided a valuable end-to-end replication, it led to issues because of the massively increased sample load. Given the manual extraction process employed by the KGH team, they were not able to process extractions at the rate they were coming in, while sharing the extraction hood. The Metabiota team has subsequently moved to manual extractions, exacerbating the issue further.

Given the space and resource constraints, the two teams have moved to a single extraction process beginning on July 11, 2014; samples are now extracted by a rotation technician from USAMRIID, and the extraction is split into two aliquots – one for KGH and one for Metabiota diagnosis. This has freed up hood space so that both teams can more readily test samples in and prevent delays due to heavy sample processing workflows.

6.0 PCR-based Diagnosis

The following sections describe in detail the PCR assays currently being used at the KGH Laboratory, including validation, controls, evidence-based implementation, and determination of positive EVD cases. As mentioned previously, the KGH Laboratory and Metabiota, using different assays, perform diagnosis on suspected EVD cases concurrently, using traditional and real-time PCR technologies. These PCR tests are carried out in the main laboratory in the Extraction/PCR biosafety cabinet (Figure 1 and 2).

6.1 Benefits of Dual Testing

The two PCR tests (traditional PCR for KGH and qPCR for Metabiota) are both integral to offering accurate diagnosis of suspected EVD cases coming to Kenema.

Traditional PCR

1. Traditional PCR technology is easier to maintain and implement in field settings, and is a standard for sustainable technologies implemented in field laboratories.
2. While not as sensitive as probe-based real-time PCR for identifying low viremia, it allows for detection of divergent viral strains.
3. It is better for long-term implementation and surveillance because it can be easily run and maintained by local personnel.

Real-Time PCR (qPCR)

1. qPCR is quantitative, which allows the technician to estimate the number of viral particles in the patient's blood.
2. It is more sensitive, which allows it to pick up positive cases with low viremia, potentially catching cases earlier than traditional PCR. However this is also more likely to introduce false positives if a low level of contamination is present.
3. It is often better for short term-implementation (as in an outbreak scenario), but more difficult to implement for long-term surveillance.

The assays are complementary and both serve important roles; using one alone can lead to false positives or negatives (see section 7.0 below). It is also important to build redundancy into the diagnostic system because of the unusual demands of biological reagents and protective equipment needed during an outbreak setting. As the outbreak has progressed in Sierra Leone, the volume of reagents and supplies needed has risen accordingly, placing unprecedented challenges on maintaining the laboratory supply stocks. If at any point, one team cannot operate, there is a back up option so that diagnostics can continue.

6.2 VHFC Traditional PCR

In late March 2014, Dr. Kristian Andersen and Mr. Stephen Gire, on behalf of VHFC and supported by the MoHS, helped the KGH Laboratory implement traditional PCR-based diagnostics using PCR platforms already utilized for diagnosis of LASV. A total of three EBOV-specific assays were tested at the KGH Laboratory and validated to assess which assays performed the best for diagnosis of EVD in Sierra Leone. Two of the PCR assays were adapted from published primer sets (Panning et al., 2007 – referred to as *FiloA/B*; Adrienne et al., 2010 – referred to as *modified Kulesh*). *FiloA/B* is a traditional or qPCR based assay, while the modified Kulesh assay was a probe-based qPCR assay that was adapted to traditional PCR by omitting the probe. These two assays were run along with an assay designed at Harvard University (referred to as KGH primer set) using new sequence information from the Guinean outbreak to determine the best primers on previously published EBOV sequences (Baize et al., 2014). This assay was not specifically designed for the Guinean outbreak, however, as Guinean EBOV sequences were not yet available.

FiloA/B primers:

FWD: 5' - AAGCATTTCTAGCAATATGATGGT -3'

RVS: 5' - ATGTGGTGGGTATAATAATCACTGACATG -3'

Modified Kulesh primers:

FWD: 5' - CTGACATGGATTACCACAAGATC -3'

RVS: 5' - GGATGACTCTTTGCCGAACAATC -3'

KGH primers:

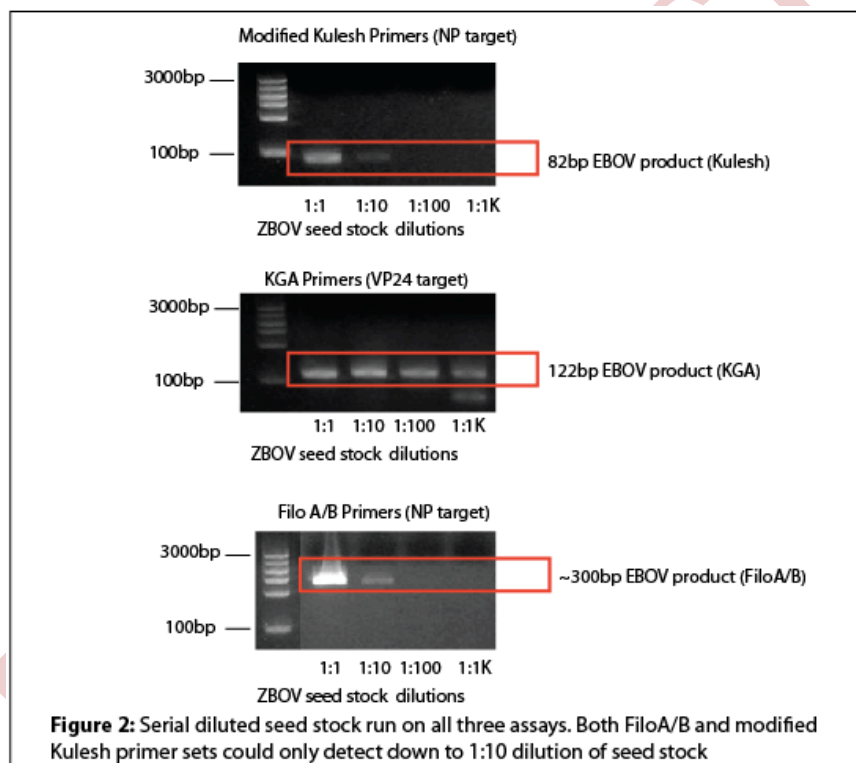
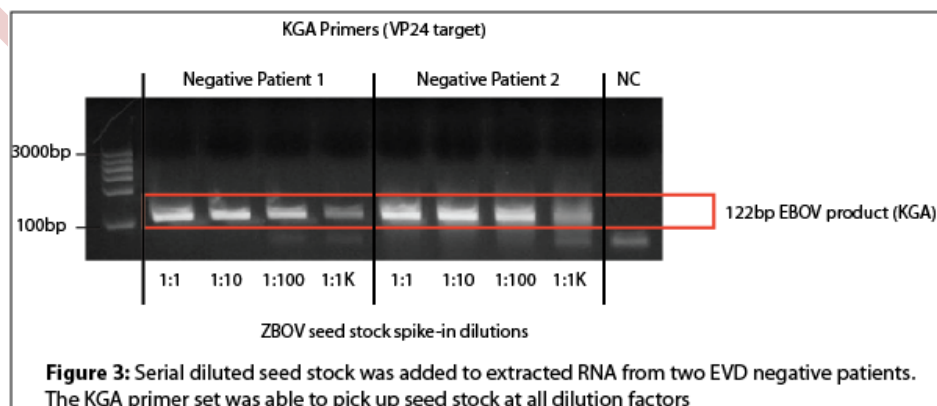
FWD: 5' - GTCGTTCCAACAATCGAGCG -3'

RVS: 5' - CGTCCCGTAGCTTTRGCCAT -3'

6.2.1 PCR Validation

These three EBOV primer sets were tested in KGH Laboratory on inactivated EBOV seed stock obtained from USAMRIID. Initially, seed stock was serially diluted from 1:1 to 1:1000, and 2µl were inputted into PCR reactions, using Invitrogen's ssIII one-step RT-PCR HiFi kit, along with a negative RNA extraction control. Both the FiloA/B and Modified Kulesh primer sets could only detect EBOV seed stock at a dilution of 1:10, while the KGH primer set could readily detect EBOV seed stock at a 1:1000 dilution (Figure 2). The FiloA/B PCR produced a brighter fragment upon gel-electrophoresis than the modified Kulesh primer set (Figure 2).

We also spiked EBOV seed stock into patient samples in order to mimic patient sample conditions (at that time, no EVD patient samples were available in Sierra Leone). As previously observed, the KGH primer set was able to detect seed stock at a 1:1000x dilution in patient extracted RNA (Figure 3), while the other two published primer sets could only detect down to a 1:10 dilution (data not shown).



Because of these results, we eliminated the modified Kulesh primer set from the PCR panel used for the diagnosis of EVD, in order to help streamline the process and decrease the diagnostic workload. The final KGH EBOV assay panel includes the KGH primer assay and the FiloA/B assay. The finalized assay panel was run according to the following cycling conditions:

Reaction Conditions				
Step 1	60°C	20 min		
Step 2	94°C	5 min		
Step 3	94°C	15 sec		35 cycles
	58°C	15 sec		
	68°C	15 sec		
	68°C	2 min		
Step 4	68°C	2 min		

Table 1. Conditions of finalized assay panel run by the KGH Laboratory.

6.2.2 Controls

Control Assays:

Included in the KGH EBOV assay were an actin assay and a No-Primer control assay. The actin control assay evaluates the success of RNA extractions. A No-Primer control assay was determined to be essential in the panel because some samples have non-specific primer-independent amplification that can often be confused with a true positive result. We believe that these samples have contaminated gDNA that self-prime. By performing a no-primer control, we are able to eliminate these samples from being reported as false positives on the KGH EBOV assay.

The finalized KGH EBOV panel was comprised of the KGH primer assay, a LASV assay, the FiloA/B assay, an actin assay, and a no-primer control assay.

Control Samples:

Both negative and positive EVD controls are used for every assay panel run at the KGH Laboratory. For the negative control, a water sample is used at the start of the extraction process (an extraction control) for each batch of extractions. This is used as the negative control for each sample tested in that batch. During initial surveillance in March and April, the KGH Laboratory used a positive control amplicon based on the specific EBOV assay, as well as EBOV seed stock from USAMRIID. However, as the outbreak has continued and positive EVD cases have been diagnosed, confirmed positive patient samples are now used as the definitive positive control for both EBOV assays in the panel, since they offer the best positive control and are readily available.

6.2.3 PCR Assay Panel Refinement

Due to the heavy burden of suspected samples, and in order to provide timely results for confirmation and reporting, several modifications were made to the assay panel in order to better handle patient volume. For the same reason RNA extractions were also streamlined, as described previously, and one sample aliquot was extracted and split between KGH and Metabiota. To date, the KGH Laboratory has tested over 800 patient samples in less than two months. Running each sample on five separate assays became too laborious and costly to maintain during the outbreak. Below we outline the modifications to the assay panel and the reasoning and need behind these modifications.

Assay Panel Modifications:

1. The LASV and actin control assays were both removed from the panel. While extraction control is important, we eliminated the actin assay in our panel because that assay is already being performed by Metabiota. The LASV assay was also eliminated because Lassa antigen ELISA is performed on all negative EBOV patient samples, and it was therefore not needed.
2. The published FiloA/B assay was removed from the panel in order to increase sample throughput. Not only did this assay not perform well in our validations, but Metabiota is already providing a second PCR testing; a third confirmatory PCR is redundant and unnecessarily drains reagent resources and personnel time.

The current KGH EBOV assay panel includes the KGH primer assay and the No-Primer control assay. This allows the KGH Laboratory to accurately diagnose patient samples and has eliminated false positives in the KGH EBOV assay. It also provides a complementary assay to Metabiota's qPCR assay.

7.0 Result Reporting

Upon diagnosis of the first EVD patient at KGH, a reporting form was created to compile results for each patient; it lists results for the KGH EBOV PCR assay, Metabiota qPCR, and a Metabiota antigen-capture ELISA, as well as any follow-up results in the case of discordant results. The agreed-upon procedure for relaying diagnostic results from the laboratory was that both Mr. Goba, as Director of the KGH Laboratory, and Dr. Wauquier, for Metabiota, were to enter results on the data sheet, which was to be compiled and signed off by Dr. S. Humarr Khan, Director of the VHF program at KGH. For any cases for which the two results were discordant, each team would repeat the process to reconcile them before official reporting. In the case of discordant results, the results would be treated internally as positive (due to the importance of timely response) until the follow up testing is complete.

This system of reporting unfortunately has not been followed. While comparison of the two PCR tests is supposed to be performed before a final determination and report to the MoHS is made on suspected cases, Dr. Kahn and Mr. Augustine Goba are often bypassed as part of the reporting system. This means that often a determination is made using only results from Metabiota's qPCR assay, bypassing KGH results. The KGH team has expressed serious concerns about this, and we hope this is corrected going forward.

While the two assays are working consistently, with only 4 discordant results in over 800 samples tested, even the few discordances show the essential role each plays in diagnosis. In all four discordant cases, the sample tested positive by Metabiota and negative by the KGH Laboratory. Two of the samples that were negative by KGH were determined to have low copy number by the Metabiota qPCR assay. This is consistent with the fact that qPCR is more sensitive than traditional PCR and is better able to detect samples with low viremia. The other two cases were eventually determined to be false positives. Thus the KGH EBOV assay seems to perform slightly better for eliminating false positives, while the Metabiota assay is better at detecting low copy number cases. Given the importance of minimizing both false

positives and false negatives in this situation, the two assays thus both play important roles in diagnosis.

Reporting of false positive results can be significantly minimized by incorporating the results of both assays in the determination. We have thus advocated that the KGH results not be bypassed in the future:

1. In the first false positive case, a sample from Port Loko came up positive by the Metabiota qPCR assay and negative by the KGH EBOV assay. The KGH team performed a repeat test that was also negative. However, the woman was brought to the KGH EVD/LF Ward and spent five days with her partner (who was a confirmed case of EVD). She was finally released when her husband survived and a repeat test result by Metabiota showed that she had been a false positive.
2. In the second false positive case, a man with no known prior contact with EVD patients from Makeni, which has no prior cases of EVD, was suspected to have EVD after becoming sick with bloody diarrhea. A sample was sent to KGH for testing and came up as a low positive on the Metabiota qPCR assay and negative for the KGH EBOV assay. The test was then repeated with identical results. The case ended up being reported as positive instead of indeterminate. Finally, the test was repeated a third time, and it came up negative by both the Metabiota qPCR assay and the KGH EBOV assay. It was then ruled to be a contamination by the Metabiota staff. This case has become a political issue since the subject was from an area that had not previously had EVD.

8.0 Concurrent Efforts

While VHFC has been performing research at KGH for nearly ten years on LF, we have suspended all research on site given the severity of the outbreak. We have, however, been aiding in diagnosis of EVD as well as LF, and sending samples to the US for sequencing. Here we describe those efforts in brief.

- Because KGH is a central referring and diagnosis center for LF, LASV diagnosis continues despite the EVD outbreak, but only for individuals that test negative for EVD. This effort is critical as LF is endemic in this region and highly fatal, and early diagnostic can affect outcome. LF diagnosis involves a rapid antigen test as well as LASV-specific ELISAs (antigen, IgM, IgG). As noted above, these antibody-based diagnostics are performed in a biosafety hood on patient samples that present as suspected VHF cases but test negative for EVD.
- In order to provide complementary support during the EVD outbreak, the MoHS approved VHFC team members from Harvard University and the Broad Institute to sequence samples from suspected and confirmed EVD cases in Sierra Leone and to compare with basic de-identified epidemiological information. The first set of samples sent to Harvard for sequencing by KGH contained 49 suspected EVD cases, of which 14 were confirmed positive for EBOV by PCR performed at Harvard. The EBOV sequences from these samples were then submitted to NCBI databases (PopSet 661348595) in order to provide the MoHS valuable data on the outbreak and to make

the data available for the scientific community to begin analyzing and providing new insights into the current EVD outbreak. Findings include:

- The current diagnostics accurately capture EVD case status in the first wave of samples. All samples positive by PCR were positive by sequencing. All samples negative by PCR were negative by sequencing.
 - The Sierra Leonean EBOV strains are very similar to the ones earlier in the outbreak from Guinea, suggesting that human-to-human transmission is likely to be the main route of infection. We do not have any data to suggest that multiple introductions of EBOV have occurred over the current outbreak, although our data is currently limited in scope to fully address this question.
 - The current outbreak strains are very close (~97% identical) to previous strains of EBOV that have caused outbreaks since the 1970s in Gabon, Congo and the DRC.
- Aliquots of samples from unadulterated plasma and from buffy coat in trizol are being stored for potential future shipments to BL-4 labs and collaborator institutions.

9.0 Short Term Recommendations

We believe that KGH laboratory is positioned to respond diagnostically to the EVD outbreak, with two long-standing partners performing complementary assays on-site. With the every-increasing case load, however, resources (diagnostic reagents, PPE) and manpower are desperately needed at the site. Because of our longstanding partnership with KGH we will continue to provide whatever support we can, but as lab scientists we look to follow the continued leadership of WHO, MSF, CDC, and Sierra Leone in responding to the crisis.

For our part, we will aim to help in any way we can to continually enhance diagnostics at the site, and provide any supplies and resources we can make available. We would also like to put forward for your consideration a few recommendations for best leveraging these resources to enhance practices at the site:

- The availability of two distinct diagnostics by two separate teams is a tremendous resource that should be fully utilized in order to get the most accurate results and response. We recommend the agreed upon protocol for reporting be reinstated -- that results from both KGH and Metabiota assays be considered and nothing be officially reported before data from both teams is compared and discrepancies are reconciled. We also recommend that Dr. Khan and Mr. Goba no longer be bypassed in the reporting structure. This has important implications for accurate reporting and outbreak response, and can minimize situations where false positives could be reported.
- While it is important to quickly respond to EVD patients, EVD patients with discordant results should be treated distinctly. We propose the individual should be listed as indeterminate for the hospital and for the MoHS until a final determination is made. We further propose isolating the patient from both healthy individuals and EVD patients, holding them in a special treatment area. There they can be cared for as if they have EVD but away from confirmed cases, until a final case determination can be made

based on retesting. In this way we can protect both the individual, who may not be infected with EBOV, and the population, in case the patient actually is positive.

- Given the heavy case load, reagents, resources, and time in the hood can become limited. We recommend following the single extraction process in the separate extraction hood, although we note that without a parallel extraction process, greater care is needed to ensure that no issues arise in extraction. If at any time problems occur with either of the two assays (e.g. due to limitations in reagents or troubleshooting of machinery), we would recommend that two PCR replicates be performed using the working assay, if it is possible.
- If it is true that Metabiota is culturing PBMC from confirmed EVD patients, we propose this be stopped immediately, given the high biosafety concerns surrounding the culture of live EBOV, as well as the issues with false positives that potential laboratory aerosols may generate.

10.0 Long Term Efforts

Unfortunately, EVD is likely to be in West Africa for some time, and we have an obligation to think of long-term ways of improving our procedures and resources. There are already a number of ongoing activities that can be leveraged to develop preparedness. We are including these here to inform you of ongoing activities and make you aware of opportunities for the future, once the outbreak subsides.

10.1 Support to complete the Lassa Ward

KGH currently maintains a year-round 25-bed ward for the care of patients with LF, where up to 600 suspected cases are seen annually. The ward is staffed with a full-time team of doctors, nurses, and cleaners. The staff has extensive training and experience treating and caring for LF patients and the majority have over a decade of experience with LF patients. A new 48-bed Lassa Ward that will replace this historic, but timeworn facility (Fig. 4B-C), but it is still in progress. Funding for the new Lassa Ward was provided by from the Naval Facilities Engineering Command (NAVFAC), heading the medical diplomacy missions of the US Department of Defense, with technical support from Tulane University and VHFC. The new ward will contain offices, a pharmacy, medical records room as well as high and low containment wards. The rooms were specifically designed to deal with infection control issues associated with VHF. Layout of the facility and example of current progress (middle panels) and details of the construction plan (lower panel) were developed with technical assistance from Jason Moses (Jason Moses Projects, NYC) and the VHFC.

Given that the LF ward has not been sufficient to manage up to 80 EVD cases at a time at KGH, and the new facility is not complete, temporary wards have had to be created to handle the patient load (Fig. 4A). Once this EVD outbreak subsides, it will be important to complete this facility to prepare for future events. It will also be important to ensure that the new ward is at a standard sufficient for both LF and EVD patients.

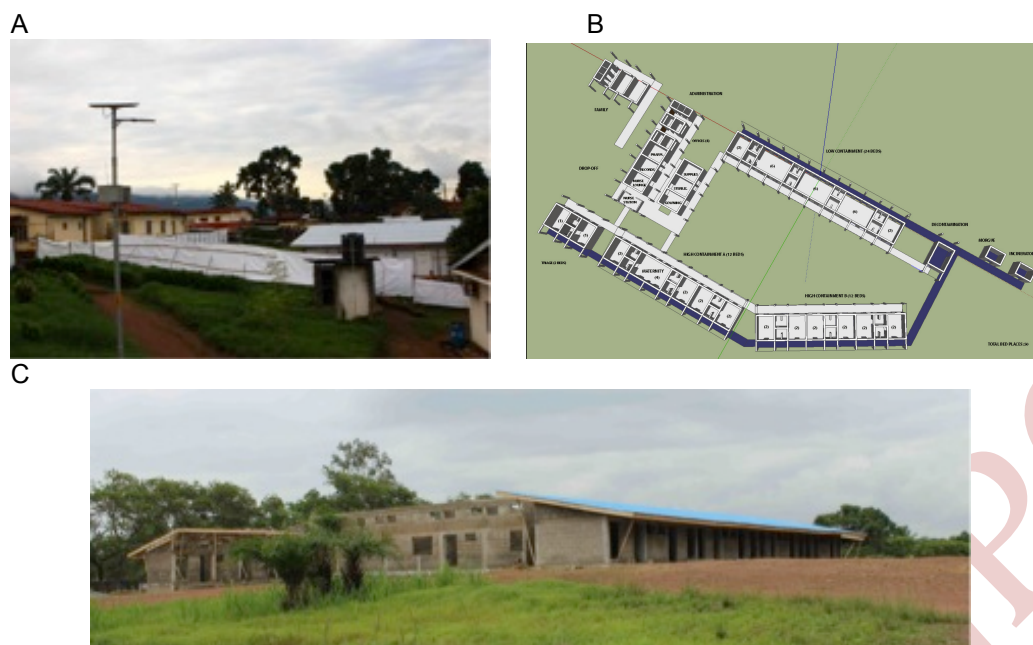


Fig. 4. The Lassa and temporary EVD Wards at KGH. (A) The current Lassa Ward is a 25-bed facility but cannot handle all EVD cases during the outbreak, and thus a temporary EVD patient ward has been constructed between the existing ward and laboratory (B-C). This facility will be replaced with a much-needed 48-bed facility (funded by NAVFAC) with construction by Fajaha International Construction..

10.2 Enhanced support for Lassa Outreach Teams

The LF outreach team at KGH is a highly experienced group of community outreach workers. The team is heavily integrated into Sierra Leone's highly structured health surveillance system, working in close partnership with district medical and surveillance officers. The outreach team conducts country-wide LF case investigations, which also include contact tracing and updating local stakeholders. The team is heavily involved in community education for the prevention and early detection of LF; in consequence, it has been tasked with outreach activities as part of the response to the ongoing EVD outbreak. The team is well versed in survey methods, including sampling strategies, questionnaire administration, and collection of blood and other specimens in community settings, and their knowledge of the communities in eastern Sierra Leone is extensive.

During the outbreak, the demands on the outreach team have increased dramatically, and safety and political risks have also escalated greatly, affecting its ability to operate effectively. It will be important to have greater support to carry out education and outreach in EVD outbreak centers and in training others in outreach nationwide for LF and EVD.

10.3 Genomics training programs (ACEGID)

The VHFC also has long-term educational commitments to the development of a critical mass of well-trained African scientists. Dr. Christian Happi of the VHFC in collaboration with VHFC partners has recently established the African Center of Excellence for the Genomics of Infectious Disease (ACEGID; www.acegid.org), centered at Redeemer's University, Nigeria

and led by collaborating. ACEGID and its partners in Nigeria, Sierra Leone, Senegal, and the U.S., through support from the World Bank and the NIH H3 Africa program, are committed to the development of training and capacity building in Africa in the field of genomics and infectious disease. ACEGID is launching both long-term graduate-level education programs for African university graduates and short-term foundational training programs for African researchers and educators. The inaugural genomics foundational training program for educators is currently being held at Harvard University; it follows a training-of-trainers model covering the topics of genomics technical procedures, diagnostics, and fundamental microbiology concepts. They also learn teaching pedagogy so that trainees can become trainers themselves in their home country. The summer 2014 program is hosting 11 students from four different institutions in Nigeria and Senegal.

While KGH and the University of Sierra Leone collaborators were invited and intending to participate in the summer genomics training program at Harvard, the EVD outbreak has necessitated their commitment to the outbreak response and a rescheduling of their participation following the outbreak. Once the outbreak subsides, we hope to have partners from Sierra Leone attend trainings in the US and in Sierra Leone to enhance diagnostic training in country, which will now include training for EVD.

11.0 Conclusions

The EVD outbreak ongoing in West Africa is of unprecedented scale, and has had a devastating impact on many sites, including KGH in Sierra Leone, where we have worked for many years and where we have many close colleagues and friends. The response to the outbreak is now our highest priority, and we are hopeful that this document can be helpful in continually enhancing the response. We look forward to working with the WHO, CDC, MSF, the Sierra Leone MoHS and other international partners in this important effort to combat the spread of EVD in West Africa.

12.0 References

1. Trombley AR, Wachter L, Garrison J, Buckley-Beason VA, Jahrling J, Hensley LE, Schoepp RJ, Norwood DA, Goba A, Fair JN, Kulesh DA. Comprehensive Panel of Real-Time TaqMan™ Polymerase Chain Reaction Assays for Detection and Absolute Quantification of Filoviruses, Arenaviruses, and New World Hantaviruses. *Am J Trop Med Hyg*. 2010 May; 82(5): 954–960. doi: 10.4269/ajtmh.2010.09-0636
2. Baize S, Pannetier D, Oestereich L, Rieger T, Koivogui L, Magassouba N, Soropogui B, Sow MS, Keita S, De Clerck H, Tiffany A, Dominguez G, Loua M, Traoré A, Kolié M, Malano ER, Heleze E, Bocquin A, Mély S, Raoul H, Caro V, Cadar D, Gabriel M, Pahlmann M, Tappe D, Schmidt-Chanasit J, Impouma B, Diallo AK, Formenty P, Van Herp M, Günther S. Emergence of Zaire Ebola Virus Disease in Guinea - Preliminary Report. *N Engl J Med*. 2014 Apr 16.
3. Panning M, Laue T, Olschlager S, Eickmann M, Becker S, Raith S, Courbot MC, Nilsson M, Gopal R, Lundkvist A, Caro Ad, Brown D, Meyer H, Lloyd G, Kummerer BM, Gunther S, Drosten C. Diagnostic reverse-transcription polymerase chain reaction kit for filoviruses based on the strain collections of all European biosafety level 4 laboratories. *J Infect Dis*. 2007 Nov 15;196 Suppl 2:S199-204
4. Shaffer JG, Grant DS, Schieffelin JS, Boisen ML, Goba A, Hartnett JN, Levy DC, Yenni RE, Moses LM, Fullah M, Momoh M, Fonnies M, Fonnies R, Kanneh L, Koroma VJ, Kargbo K, Ottomassathien D, Muncy IJ, Jones AB, Illick MM, Kulakosky PC, Haislip AM, Bishop CM, Elliot DH, Brown BL, Zhu H, Hastie KM, Andersen KG, Gire SK, Tabrizi S, Tariyal R, Stremlau M, Matschiner A, Sampey DB, Spence JS, Cross RW, Geisbert JB, Folarin OA, Happi CT, Pitts KR, Geske FJ, Geisbert TW, Saphire EO, Robinson JE, Wilson RB, Sabeti PC, Henderson LA, Khan SH, Bausch DG, Branco LM, Garry RF; Viral Hemorrhagic Fever Consortium. (2014). Lassa fever in post-conflict Sierra Leone. *PLoS Negl Trop Dis*. Mar 20;8(3):e2748. doi: 10.1371/journal.pntd.0002748. PMID: PMC3961205