Persistence of Ebola Virus in Body Fluids of Ebola Virus Disease Survivors in Sierra Leone

Request for OMB Approval for a New Information Collection Request

Supporting Statement B

Collections of Information Employing Statistical Methods

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Part B — Collections of Information Employing Statistical Methods

1. Respondent Universe and Sampling Methods

1.1 Respondent Universe

This research study will enroll individuals with documented previous Ebola virus (EBOV) infection from two study sites in two high EVD burden districts in Sierra Leone (Western and Port Loko). Ebola virus disease (EVD) survivors at different points of convalescence, ranging from the time of discharge from an Ebola Treatment Unit (ETU) up to 30 weeks of convalescence, will be targeted for enrollment.

Eligible participants are adults (18 years of age or older) and must demonstrate an EVD discharge certificate and a national identification card in order to be recruited. In order to ensure survivor status and eligibility for the study, results may be cross-checked against the national laboratory database. Eligibility for each component of the study is as follows:

- **Pilot —** Adult male (18 years or above) survivors will be enrolled if eligible, with assistance of the survivors association in Freetown and the collaborating hospital and clinic.
- **Main Study —** All adult men or women (18 years or above), discharged from an ETU or selected from survivor registries with a certificate of recovery, are eligible.

1.2 Sample Size

As this project will be conducted in the midst of an ongoing public health response, sample sizes may be adjusted by the Sierra Leone Ministry of Health and Sanitation (MoHS) and the Ministry of Social Welfare, Gender, and Children's Affairs (MSWGCA) in accordance with national and local capacity and in a manner such that they will not interfere with response efforts. The number of participants will depend largely upon the number of EVD survivors from the selected clinic sites who can be located and agree to provide body fluid specimens.

Sample sizes for each component of the study are as follows:

- **Pilot —** A maximum of 100 patients will be included with a target of 40% between 2 and 3 months convalescence period and 60% with over 3 months convalescence period.
- **Main Study —** There are three target groups to be enrolled in the main study:
    - 240 participants (120 male + 120 female) will be recruited at varying points post discharge, but recent survivors (0-3 months) will be prioritized. No more than 10 patients of a single gender shall be recruited into a single week
        - At least 60 menstrual samples will be collected from the 120 women recruited. If any of the women enrolled are lactating, breast milk will also be collected
    - 30 People living with HIV and are EVD survivors will be recruited.

If the number of patients discharged from ETUs per week is no longer sufficient to meet study sample sizes (i.e., by the time the Main study is implemented), it is anticipated that there will be adequate numbers of potential participants in survivor registries to meet the study sample sizes.

For the primary outcome of live virus isolated in tissue culture, even one positive result is of interest; as such a finding would be expected to have important implications for guidance to future caregivers, patients, and their families. Similarly, we would not want to conclude that there is no probability of persistent live EBOV virus in the fluids we test if, in reality (within the larger population), there is. Therefore, the primary power calculation of interest is the degree of confidence we can have in a null (no positives) result from our tissue culture testing. In other words, we need to know the upper confidence limit for a calculated proportion of 0% in our study.

There are several ways to calculate an upper bound for a 95% confidence interval for a 0% finding. The rule of thumb for the upper bound is 3/N, where N is the number of specimens tested. This method is accurate when the sample is sufficiently large. Calculating the upper bound precisely requires use of the binomial distribution. The table below lists the upper bounds for a 1-sided 95% confidence interval estimated using Stata SE V12.1:

Numerator	0	0	0	0
Denominator	50	100	200	300
Upper bound	7.1%	3.6%	1.8%	1.2%
Lower bound	0%	0%	0%	0%

1.3 Sampling Method

Survivor associations in each of the districts will be involved with the study implementation. A small group of survivors will be formed to serve as key informants for sensitization of the community and recruitment for the study.

In collaboration with the survivors association and the MSWGCA, a list of established EVD survivors will be selected in accordance with sample size criteria. These groups of established survivors will be invited to a meeting in their district where they will be informed about the study process and benefits by MSWGCA staff and/or survivor key informants. The interested participants will be asked to come in for an appointment where they will review and sign the informed consent with trained study staff, In addition, print and radio advertisements may be used to recruit for the study.

Pilot

The recruitment of survivors for the pilot will be done on a voluntary basis with the help of the association of survivors in Freetown and before the samples for the Main Study are selected. A collaboration with the national survivors' association has already been started, and the organization has been part of the formative work. The association foresees no challenges in recruiting participants for the pilot study.

Main Study

Recruitment from the ETUs	All adult patients newly discharged from an ETU during the recruitment period will be asked to participate in the study
Recruitment from survivor registry	Survivors will be contacted according to their duration of convalescence and their place of residence.
Recruitment from other sources	If the number of patients is not enough, an open cohort process where participants will be recruited by using local ETU records, survivor networks and services; community outreach, including radio solicitations, may also be considered. People living with HIV (PLHIV) who also are Ebola survivors will be invited to participate in a sub-cohort. The "network of HIV positives in Sierra Leone" (NETHIPS) and local networks who work with the PLHIV will be used to identify and reach out to participants. HIV counselors will also encourage their clients who are EVD survivors to participate in the study.

2. Procedures for the Collection of Information

2.1 Sampling Plan

The Sierra Leone MoHS will ensure coordination of project activities with routine MoHS response activities. Data from routine MoHS response activities (case investigation and contact tracing) will be shared for project purposes as needed. The Sierra Leone MoHS will advise in hiring local clinical and data entry staff. Clinical staff will interact with participants; clinical and data entry staff will have access to individually identifiable data. CDC and WHO will provide technical assistance in all project aspects and will supervise clinical staff and lead data analysis. As supervisory staff, CDC and WHO field staff will interact with participants and have access to individually identifiable data.

The MoHS identified two study sites in two high EVD burden districts (Western and Port Loko) for the study:

- M34 hospital, Freetown (pilot site location)

- Lungi Government Hospital , Port Loko

For the pilot study and the main study, the study site(s) will be the setting for all initial and follow-up visits of survivor participants, including interviews, specimen collection, and counselling regarding test results. Study participants can be referred to HIV and survivor services within the hospitals where the study sites are located.

For the study:

- Eligible study participants must be adults (>18) and demonstrate an EVD discharge certificate and a national identification card in order to be recruited. To ensure survivor status and eligibility for the study, collaborators may cross-check laboratory information as available. This information will be recorded in the Intake Form (**Attachment 1**) and a unique ID will be assigned.
- Survivors with experiences of experimental treatment during their EVD as well as any pregnant women will be included in the study
- Entry will then begin with the informed Consent Form. An additional consent form, or re-consent form, has been created that includes consent to participant in a 3 and 6 month follow-up visit and storage of any virus isolates that may come from a sample. (**Attachment 2a, 2c/2b, 2d**; for pilot study, Main study/3 and 6 month follow-up)
- A Participant Study ID Card (**Attachment 3**) will be issued to the participant for verification of identity at subsequent study visits.
- The participant will then complete a Survivor Questionnaire (**Attachment 4a and 4b**) and initial specimen collection.
- Specimen(s) will be tested for EBOV RNA by RT-PCR in Sierra Leone at the CDC laboratory facility in Bo and the China CDC laboratory in Jui; any specimen positive for EBOV by RT-PCR will be frozen and shipped to CDC for culture/virus isolation in a BSL-4 laboratory facility in Atlanta, GA.
- Participants with a RT-PCR positive result in any of the initial specimens will be invited to complete a Survivor Follow-up Questionnaire (**Attachment 5a and 5b**) at every subsequent study visit and to provide prospective specimens (i.e., repeat specimen collection) of each RT-PCR positive body fluid according to the following schedule:
    - Semen collection every 2 weeks until the RT-PCR is negative (pilot).
    - Specimen (i.e., semen or vaginal secretions, rectal swabs, sweat, urine, saliva, tears, menstrual swabs, breast milk) collection will be scheduled for every 2 weeks until two consecutive test results are RT-PCR negative (Main study)
    - At 3 and 6 months after initial discharge, participants will be asked to come in to complete a 3 and 6 month questionnaire (**Attachment 8a and 8b**) give an additional sample. They will be given their results two weeks later and if negative at the 3 month visit, will not be contacted again until 6 months. If positive at 3 months or 6 months, they will be asked to return every two weeks until receiving two consecutive negative tests following normal sampling procedures.
- All laboratory results will be entered into a Laboratory Results Form (**Attachment 6**) marked with the participant's study ID and kept in confidential storage for entering of

follow-up results.

- At follow up visits, laboratory results will be shared with the participant, and transmission prevention and potentially other counselling messages will be provided via a Counselling Script (**Attachment 7a and 7b**).
- Confirmatory virus culture results will be reported back to participants when available.
- Once a participant has had two consecutive negative test results for all body fluid specimens collected in this study, there will be no further follow-up.

2.2 Specimen Transportation, Laboratory Testing, and Storage

At the study sites

- Semen samples from the Pilot study were transported from the study site to the CDC Bo laboratory three times weekly during the pilot study until Oct. 17, 2015. Starting Oct. 20, 2015, semen samples from the pilot will be transported on the same 3 day/week schedule to China CDC's laboratory in Jui, Western Rural.
- Samples to be collected in the Main study will be transported daily to China CDC's laboratory in Jui.

All sampling and handling of specimens collected for transportation should follow procedures for labeling, packaging, transporting, and storing bio-hazardous material.

At CDC Bo and China CDC laboratory:

- RT-PCR will be performed on all specimens for two viral targets, as well as an internal human control, human beta-2-microglobulin (B2M), according to standard guidelines.
    - Targets in CDC Bo's assay: viral matrix protein 40 (VP40) and nucleoprotein (NP),
    - Targets in China CDC's assay: glycoprotein (GP) and nucleoprotein (NP)
- Validation was performed over several weeks to ensure there was adequate matching of results between the two labs on aliquots from the same sample.
- If the internal human control is negative for a given specimen, this would be viewed as an inadequate sample and a new specimen would be requested.
- Specimens will be stored at -80C and those that are RT-PCR positive at the CDC Bo or China CDC laboratory will be shipped to the CDC-Atlanta BSL-4 laboratory for virus isolation testing, following appropriate protocols for transporting Category I biohazards.

CDC Atlanta BSL-4 laboratories:

- In order to determine if a given body fluid specimen has live virus and is potentially infectious, virus culture will be performed. This can only be safely performed in Biosafety Level (BSL)-4 laboratory conditions, which are not available in Sierra Leone. Therefore specimens which have detectable EBOV RNA by RT-PCR will be transported frozen with adequate safe packaging and cold chain to Atlanta, GA, USA for virus isolation.
- Testing for live virus will be performed at the CDC BSL-4 laboratory in Atlanta, GA, USA, ideally within 6 months of specimen receipt. Standard EBOV virus isolation protocols for diagnostic specimens will be followed.
- If specimen type and volume permit, the viral titer (TCID50/mL) for the specimen will be determined. This will inform the amount of infectious virus in a specimen, unlike virus isolation which will tell you a binary (i.e., yes or no), to infectious EBOV virus in the specimen.

- Residual body fluid specimens will be destroyed. Viral isolates, if any, may be preserved at CDC for further study in collaboration with MoHS and WHO (in accordance with the research collaboration agreement). Body fluid isolates will be kept in liquid Nitrogen frozen storage (-165C) in a secure location at CDC's BSL-4 laboratory. Virus isolates can be shared to requesting parties assuming that the requesters can comply with the necessary biosafety requirements (BioSafety Level-4, Select Agent compliance). Further study of virus isolates may include genetic analysis and comparison with other virus strains.

2.3 Data Management

- All MoHS-owned data related to a study participant will be assigned the participant's unique study ID. No patient-identifying information (i.e., names) will leave Sierra Leone.
- Interview data will be organized in databases stored on secure local servers within the Ministry of Health in Sierra Leone and WHO and will be backed-up regularly. Collected data will be directly recorded on computers or tablets to minimize data recording and entry errors and minimize delays in data availability. If paper forms must be used, interview responses will be entered into the database either daily or as a group at the close of data collection; and 10% of entered forms will be re-checked to identify any problems with data entry accuracy that must be addressed.
- Electronic equipment and files will be kept password-protected.
- Paper forms and electronic devices will be kept locked when not in use.
- All individual data identifying direct patient identifiers will be removed from the dataset before analysis and replaced with a unique participant code that can be linked back to individuals via a master key at a centralized secure server and database.
- Individual records and the key linking the participant code number will be kept secure, accessible only to the local study team under the supervision of the study PI and MoHS in collaboration with WHO and CDC.
- Paper interview forms, if used, will be destroyed within one year after all data are entered and verified.
- Laboratory results will be batch processed and complete PCR results for all specimen types will be reported back within 1 week of specimen receipt to each site coordinator. A positive RT-PCR result on any specimen should be entered in the secured data base and reported within 8 hours to the study PI and WHO and CDC local coordinators as well as the site coordinator where the specimen was collected. Laboratory staff will identify specimens only by the labelled study ID, and will not have access to any personally identifying information.
- Site coordinators will provide laboratory results only in person and only to participants who (1) return for a follow-up study visit; and (2) present their study ID card or confirm their identity and study ID number; and (3) request to receive their own results. Individual results will not be shared with anyone other than the study participant. Results will be presented according to the counselling script in Attachment 8.
- Viral isolation results from CDC Atlanta will be reported promptly to the study PI and entered in the secured data base as they arrive. These confirmatory results will not be

available within a clinically relevant time frame (6 months) although positive results will be reported to participants.

3. Methods to Maximize Response Rates and Deal with Nonresponse

As previously noted, survivor associations in each of the districts will be involved with the study implementation. A small group of survivors will be formed to serve as key informants for sensitization of the community and recruitment for the study. Study participation is voluntary, and study leads will make every effort to maximize the rate of response. Additionally, support will be offered to every participant at every visit including: specific EBOV transmission prevention counselling and provision of condoms distributed by trained counsellors; voluntary human immunodeficiency virus (HIV) testing and counselling will be offered to all participants, and HIV-positive study participants will be referred for HIV counselling and treatment services; participants in need of specific health services will be referred to the appropriate clinic/hospital for care and management. When pertinent, counselling on potential EBOV transmission via breastfeeding will be given by trained counsellors and alternative feeding will be provided. The counselling script (**Attachment 7a and 7b**) address many anticipated study participant questions. Finally, several survivors' support groups are actively working to provide counselling and support of various qualities to EVD survivors in Sierra Leone, in collaboration with UNICEF and the national survivors' association, which study collaborators anticipate will mitigate community concerns.

Main challenges to the success of the project include willingness of participants to provide biological specimens from sensitive locations, and to share intimate information on sexual behavior. Proposed solutions are to ensure trained interviewers and medical staff of appropriate genders and cultural backgrounds, who are used to discussing sexual behavior, and to take bio-samples of sensitive character. Also to secure an environment where the interviewee will be secure the procedures and information is kept confidential. The ability to provide quick turnaround of specimen processing and analysis for timely report back to participants.

Every effort will be given to maintaining communication with enrolled participants. Contact information of the participant will be collected and contact information for local study staff will be provided at enrollment.

4. Test of Procedures or Methods to be Undertaken

Using WHO funds only, the study team previously held a focus group with 15 male EVD survivors at the Military 34 hospital grounds; CDC personnel observed, but did not conduct, this focus group. The study team provided an overview of the study and explained the rationale, followed by a review of the informed consent and questionnaire with the group. The group provided feedback on their comprehension of the informed consent, the acceptability of the subject matter, and

questionnaire content. Revisions were made to the informed consent and questionnaire based on this feedback. The group did not find the informed consent or questionnaire process burdensome.

The pilot study is intended to

- Inform the process and implementation of the Main study
- Provide rapid results on the prevalence of EBOV by RT-PCR in semen of survivors
- Assess the prevalence of live Ebola RNA detected by culture/viral isolation from RT-PCR positive semen

Only minor study changes are expected, if any. As a result, a combined approval for the pilot and the subsequent Main study is preferred. Any changes to the procedures or methods to be used will be communicated to IRB and OMB as quickly as possible before data collection for the Main study begins.

5. Individuals Consulted on Statistical Aspects and Individuals Collecting and/or Analyzing Data

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*Not Available (NA)

LIST OF APPENDICES

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APPENDIX B. 60-Day Federal Register Notice
APPENDIX C. Public Comments
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ATTACHMENT 2b. Consent Form – Main Study
ATTACHMENT 2c. Re-Consent Form – Pilot Study
ATTACHMENT 2d. Re-Consent Form – Main Study
ATTACHMENT 3. Participant Study ID Card
ATTACHMENT 4a. Survivor Questionnaire – Male
ATTACHMENT 4b. Survivor Questionnaire - Female
ATTACHMENT 5a. Survivor Follow-up Questionnaire - Male
ATTACHMENT 5b. Survivor Follow-up Questionnaire - Female
ATTACHMENT 6. Laboratory Results Form
ATTACHMENT 7a. Counselling Script – Male
ATTACHMENT 7b. Counselling Script - Female
ATTACHMENT 8a. 3-6 Month Follow-up Questionnaire – Male
ATTACHMENT 8b. 3-6 Month Follow-up Questionnaire – Female