



International meeting on persistence of Ebola
virus RNA in semen and implications for public
health

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Acronyms

AD-MA	Aggregate data meta-analysis
B2M	Beta2-microglobulin
BNi	Bernhard Nocht Institute for Tropical Medicine
BSL4	Biosafety level 4
CPES	Comprehensive Programme of Services for Ebola virus disease Survivors
Ct	Cycle threshold
EVD	Ebola virus disease
ELWA	Eternal Love Winning Africa
ETU / ETC	Ebola treatment unit / Ebola Treatment Center
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
INSERM	Institut national de la santé et de la recherche médicale
IPD-MA	Individual participant data meta-analysis
PFU	Plaque forming unit
RNA	Ribonucleic acid
RT-PCR	Reverse transcription polymerase chain reaction
SCID	Severe combined immunodeficiency
TB	Tuberculosis
TCID	Tissue culture infective dose
UNAIDS	Joint United Nations Programme on HIV/AIDS
UNC	University of North Carolina at Chapel Hill
US CDC	United States Centers for Disease Control and Prevention
WHO	World Health Organization

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Introduction

Background

The 2014–2016 Ebola virus disease (EVD) outbreak in West Africa was the largest and most complex Ebola outbreak ever seen since the virus was first discovered in 1976. The outbreak caused more cases and deaths than all others combined, and has also generated a survivor community of unprecedented size. Little is known about the long-term health impacts of Ebola virus infection on survivors, as well as the persistence of Ebola virus in the body and corresponding public health consequences.

Before 2014, Ebola virus had been detected in a limited number of semen samples from male survivors up to 101 days by PCR detection of viral RNA. Though occasionally suspected after prior outbreaks, sexual transmission of Ebola virus had not been clearly documented. At the beginning of the West Africa outbreak, WHO interim advice recommended that survivors abstain from sexual intercourse or use condoms for at least three months after recovery from Ebola virus disease to prevent sexual transmission. These recommendations were revised during the outbreak as longer-term detection of Ebola virus RNA in semen and one case of suspected sexual transmission from a survivor was described.

The evidence base has rapidly evolved over the past three years. Since 2015, ongoing survivor cohort studies in the three most affected countries (Guinea, Liberia and Sierra Leone) have been investigating the persistence and consequence of Ebola virus RNA in semen and other body fluids. In parallel, national semen testing programmes have been established in each of the affected countries to provide semen testing and counselling as part of a broader package of care to survivors. These survivor cohort studies and national semen testing programmes have data and findings that are of interest for the development of public health recommendations.

Objectives

A better understanding of how long the virus remains in body fluids other than blood and which host factors determine persistence in the body fluids is essential to shape appropriate public health guidance.

To address the concerns of survivors and the research emerging from this field, in collaboration with John Snow Incorporation, and the United States National Institutes of Health, WHO convened a meeting of principal investigators, scientists and national programme officers involved in research with Ebola survivors in Guinea, Liberia and Sierra Leone from 28-30 June 2017 in Monrovia, Liberia.

The specific objectives of the meeting were to:

- review and compare findings from survivor cohorts in Guinea, Liberia and Sierra Leone and other settings;
- review and compare findings from national semen testing programmes and other non-research settings;
- discuss the revision of public health recommendations related to sexual transmission of Ebola virus disease; and

- define a research agenda to address remaining questions and to inform research responses around viral persistence in future EVD outbreaks.

Methodology

The meeting invited participants from the Ministries of Health of the three countries affected by the Ebola 2014-16 outbreak (Guinea, Liberia and Sierra Leone), staff of national semen testing programmes, principal investigators and research staff of Ebola survivor cohort studies, representatives from Ebola virus disease survivor groups, and staff from public health agencies and WHO.

The meeting was structured into plenary sessions, discussion sessions and a working-group session. Participants were also invited to attend an optional field trip to John F. Kennedy Medical Center in Monrovia – a major national public hospital used to treat cases of Ebola virus disease during the 2014-16 outbreak and currently the centre of Ebola survivor follow-up programmes and research in Liberia.

Declarations of interest and confidentiality

46 experts from related scientific and public health fields were invited to attend the meetings. In accordance with WHO rules and regulations, all experts completed and submitted a Declaration of Interest (DOI) form before participating in the meeting. DOI forms were reviewed prior to the meeting, and no conflicts of interest were identified.

Given the sensitive nature of the ongoing research discussed during the meeting, all experts and persons in attendance also signed and submitted a Confidentiality Undertaking form prior to participating.

1. Opening session

Dr Zakari Wambai, WHO Country Office, Liberia, opened the meeting on behalf of the WHO Representative to Liberia. Dr Wambai thanked the Ministry of Health and government of Liberia for hosting the meeting, as well as the nationals and governments of the three countries affected by the 2014-16 Ebola virus disease outbreak – Guinea, Liberia and Sierra Leone – for their help to close the gaps in our knowledge of Ebola virus disease and to address the health issues of people who have survived the disease. Lastly, he thanked other partners involved in developing the body of Ebola research for their support and continued partnership, most notably Ebola survivors without whom this research would not be possible.

Dr Catherine Cooper, Assistant Minister for Curative Services, Ministry of Health, Liberia, welcomed the participants to Liberia on behalf of the Minister of Health. Dr Cooper thanked WHO, a key partner of the Ministry of Health, for convening the forum. Ebola is still a threat to West Africa and to the world. Much is still unknown about the virus and the disease, and the studies that are ongoing are gradually opening a window of knowledge. The 2014-16 Ebola virus disease outbreak was a dramatic event for West Africa, but has provided an opportunity to study and learn how to combat the virus more efficiently. The recommendations that will be developed from this research are critical to guide our future interventions.

Dr Sakoba Keita, Agence Nationale de Sécurité Sanitaire, Guinea, expressed appreciation for the convening of this meeting. It is not known how Ebola came to Guinea, or where it might currently be in the country. It is important to support every study that can lead to better knowledge of the virus. This is why Guinea has opened its doors to many medical trials, and continues to support the efforts of researchers working on Ebola.

Dr Pierre Formenty, World Health Organization, welcomed the participants and noted that this was the first meeting of its kind between the three countries affected in the 2014-16 Ebola virus disease outbreak. The meeting has brought together the major scientific teams working on Ebola, together with representatives of the survivors of the disease. This will be the start of an ongoing body of work to describe and address the issues faced by survivors, and will be of great importance to the health systems of the affected countries and to WHO.

2. Findings from cohort studies

2.1 PREVAIL III natural history study, Liberia

Mosoka Fallah, on behalf of the PREVAIL Study Group

The PREVAIL III study was initiated in June 2015 to investigate clinical sequelae, virologic persistence and immunologic consequences of acute EVD. EVD survivors and close contacts (controls) in Liberia were invited to participate in this observational study. Symptoms, physical examination findings, and laboratory results for antibody-positive survivors were compared to antibody-negative close contacts. Persistence of Ebola virus RNA in semen and other body fluids of survivors was determined in subgroups of participants.

Over 1000 Ebola survivors and 1700 close contacts were enrolled in Liberia. A subgroup of 193 male survivors provided over 800 semen samples for Ebola virus RNA analysis. The median time from acute EVD illness to first semen sampling was 19 months. A test was considered to have detectable Ebola viral RNA if either the GP or the NP gene were detected at any CT value using the GeneXpert platform. Ebola viral RNA was detected in the semen of 37% of men tested at least once. For the 178 men who provided more than one semen sample, detection of viral RNA was intermittent in 32% (no detection followed by detection or vice versa); with 20 men (28% of those with at least one positive result) having two non-detection tests followed by a detection. Ebola viral RNA was not detected in any of the semen samples collected from over 200 close contacts.

Pregnant female survivors enrolled in PREVAIL III were recruited between December 24, 2015 to January 10, 2017 into the birth cohort sub-study. The median time from acute EVD illness to enrolment was 27 months. The following samples were tested for Ebola virus RNA: cord blood, placenta tissues, vaginal swabs, maternal blood, and breast milk. Maternal and infant serum was tested for Ebola-GP specific IgG antibody levels. A total of 74 pregnant women and 77 children were enrolled into this sub-study. Thirty-nine cord blood, 39 maternal blood, 39 placenta swabs, 38 placenta tissue samples, 331 breast milk samples and 339 vaginal swabs were collected. All samples tested negative for Ebola viral RNA. Neonatal cord blood samples contained Ebola specific IgG at levels similar those observed in maternal samples. For all infants with follow-up serology samples, levels of Ebola-GP specific IgG declined in a manner consistent with transplacental transfer of maternal IgG.

2.2 PostEboGui cohort study, Guinea

Philippe Msellati, UMI 233 Institut de Recherche pour le Développement/Université de Montpellier, France

The PostEboGui cohort study is a comprehensive study of clinical, virological, immunological, genetic, psychological and social impacts of Ebola virus survival. The study was initiated in March 2015 and has enrolled 802 adult participants - around two thirds of the known survivors in Guinea. Participants receive free care and transportation costs, and an emergency social fund has been provided to support them.

The study has detected Ebola viral RNA in 0.003% of saliva samples (n=335), 0.004% of urine samples (n=530), and 0% of cervicovaginal (n=191)

and breast milk (n=14) samples. RNA has been detected in the semen of 8% of males who have provided samples, with a range in time from first symptoms to provision of sample in which RNA was detected of 29-551 days. Modelling estimated that the median time to produce an RNA undetected sample was 46.4 days after onset of symptoms and that of the 1270 Guinean patients discharged from Ebola treatment centres, two males would have Ebola RNA detectable in their semen as of 31 August 2016. Continued follow-up until viral clearance in semen is therefore needed to reduce the risk of sexual transmission from survivors, and prevention will rely on condom use.

2.3 Sierra Leone viral persistence study, Sierra Leone

Nathalie Broutet, World Health Organization; Ute Ströher, United States Centers for Disease Control and Prevention

Beginning in May 2015, the aim of the cohort study was to investigate the persistence of Ebola virus in body fluids, including semen, in male and female survivors. After a baseline visit, participants donated specimens every two weeks until two consecutive negative PCR results were obtained. Additional visits at three and six months after the second negative PCR result were scheduled for follow-up. Participants received counselling at each visit and completed questionnaires including information on their sexual activity.

Preliminary results showed that persistence in semen and other body fluids from this study confirmed results from earlier published studies. Ebola RNA was detected in 7.5% of samples one year after onset of symptoms. The longest period during which the RNA was detected in semen was found to be close to two years. These results are preliminary. Analysis of the factors associated with persistence of Ebola virus in semen is still ongoing. Additionally the study highlights important gaps in the uptake of advice on safer sex targeted at survivors. Only a small number of sexually active survivors reported consistent condom use, and less than half of survivors reported having had sexual intercourse during the immediate time period after ETU discharge.

2.4 UNC-ELWA-CRM cohort study, Liberia

William Fischer and David Wohl, University of North Carolina School of Medicine

The cohort study was initiated in August 2015 to address Ebola survivors' concerns of ongoing medical complications and fear of transmitting the disease sexually. The objectives are to better understand the clinical sequelae of Ebola virus disease; describe the duration and patterns of detection of Ebola virus RNA in the genital fluids of men and women; and to determine the infectivity of genital fluids with detectable Ebola virus RNA. A total of 330 survivors were enrolled in the study (48% men) and followed every three months with surveys and sample collection.

The Cepheid Xpert Ebola virus assay was validated for detection of Ebola virus in semen and used in the study. Semen samples from 149 male participants were analysed, with Ebola virus RNA detected in samples from 13 participants (9%). In 8% of participants, Ebola virus RNA was detected in semen more than two years after symptom onset, with the longest period of time being 965 days. In eight of the 13 participants (62%) who produced semen samples in which Ebola virus RNA was detected, the detection of RNA occurred intermittently (i.e. in non-consecutive samples, with samples taken a minimum of two weeks apart). Men with persistent Ebola virus RNA were observed to be significantly older and report more vision problems.

2.5 BNI-EU-INSERM cohort study, Guinea

Sophie Duraffour, Bernard Nocht Institute, Hamburg

The cohort study enrolled males aged 18-65 years who had been discharged from three selected Ebola treatment units in Guinea between 20 January and 6 July 2015.^{1,2} The participants were confirmed to have had Ebola virus infection by reverse transcription polymerase chain reaction (RT-PCR) and provided written informed consent. They provided samples of semen, blood and urine, were given counselling on safer sexual practices and condom use, and were followed-up every 3-6 weeks until Ebola RNA was undetectable in their samples in two consecutive visits. Twenty-six participants were included in the final analysis, of which 19 (73%) had detectable Ebola RNA in their initial semen sample (median of 55 days). Sixteen of these participants subsequently produced two consecutive samples (provided two weeks or more apart) in which Ebola RNA was undetected after a median of 158 days, two were lost to follow-up, and one continued to produce semen with detectable RNA by the last follow-up at 407 days after symptom onset.

Pilot studies conducted on severe combined immunodeficiency (SCID) mice demonstrated semen infectivity up to day 233 post-onset of symptoms (1st passage) and day 165 post-onset (2nd passage). Modelling produced from the study predicts 50% of male survivors will have detectable RNA in their semen up to 115 post-onset, and 10% will have detectable RNA in semen up to 294 days post onset.

2.6 Ebola survivor follow-up, Guinea

Axelle Ronsse, Médecins Sans Frontières, Brussels

The unprecedented scale of the 2014-16 Ebola outbreak in West Africa demonstrated the need for ongoing medical and psychological care for over 10,000 survivors. In response to this need, temporary survivor clinics in Guinea, Sierra Leone and Liberia were established by Médecins Sans Frontières from January-September 2016. The clinics were established to provide psychological support to people affected by Ebola, reduce stigma of survivors and affected people, and to provide high quality medical care for Ebola complications. In Guinea alone over nine months, around 1,850 medical and 1,000 psychological consultations were conducted for survivors and other affected persons.

Semen samples from 69 male survivors were tested to detect viral RNA. RNA was detected in two cases: a 38 year old, eleven months after Ebola treatment centre (ETC) discharge, and; a 20 year old, two months after ETC discharge. A third one was inconsistent. Semen testing was difficult to implement with survivors due to cultural issues, insufficient quantities of semen for testing, no sharing of results and uncertainty regarding patient confidentiality. Overall, the clinics demonstrated the specific needs of Ebola survivors and the importance of survivors and affected persons to be able to access psychosocial support. Follow-up of survivors should begin the day they are discharged to enable early detection of potential complications and access to health care.

¹ Sissoko, Daouda et al., 'Persistence and clearance of Ebola virus RNA from seminal fluid of Ebola virus disease survivors: a longitudinal analysis and modelling study', *The Lancet Global Health*, vol. 5, iss. 1, e80 - e88. Available at [http://www.thelancet.com/journals/langlo/article/PIIS2214-109X\(16\)30243-1/fulltext](http://www.thelancet.com/journals/langlo/article/PIIS2214-109X(16)30243-1/fulltext) (accessed 18 September 2017).

² Diallo, Boubacar et al., 'Resurgence of Ebola Virus Disease in Guinea Linked to a Survivor With Virus Persistence in Seminal Fluid for More Than 500 Days', *Clinical Infectious Diseases*, vol. 63, iss. 10, 15 November 2016, pp. 1353–1356. Available at <https://academic.oup.com/cid/article-abstract/63/10/1353/2452977/Resurgence-of-Ebola-Virus-Disease-in-Guinea-Linked?redirectedFrom=fulltext> (accessed 18 September 2017).

2.7 Discussion

Condom use: Rates of reported condom use in survivor populations are still very low – what are the risks to public health?

Recommendations to use condoms to prevent transmission of Ebola virus may be integrated into broader policies or recommendation for condoms use to protect against sexually transmitted infections including human immunodeficiency virus (HIV) and Ebola.

Infectivity: It is difficult to produce evidence of infectivity in laboratories. What are the factors that make sexual transmission happen? Looking at the literature around HIV may be helpful.

Samples: How are samples collected, stored, transported and tested? Each of these processes gives rise to additional variables that may affect tests results and their comparability. Are samples frozen or refrigerated? Are samples tested on the same day or months later? Are samples shipped in-country at room temperature or at +4°C or frozen at -20°C?

Shedding versus detection: The distinction between intermittent shedding and intermittent detection should be made clearly and the terms used more accurately. In most of the studies discussed, intermittent detection has been observed, potentially implying intermittent shedding of viral RNA.

Survivor stigmatization: How to fight against stigma? Some survivors have had to move and leave their villages. What else can be done to support the survivor community?

Testing: Important to state not just which assay is used, but which gene targets and definitions for detection versus non-detection. The variables of platform sensitivity, definitions, genes, as well as heterogeneity in frequency of testing likely explain variability in results. What are the limits of detection on the assays used?

3. Further learning from cohort studies

3.1 Using individual participant data meta-analysis (IPD-MA) to inform public health recommendation for emerging pathogens

Lauren Maxwell, World Health Organization

Meta-analysis is the combination of quantitative evidence from related studies to produce results based on the whole body of research. In individual participant data meta-analysis (IPD-MA), individual-level data is synthesized in either a one- or a two-stage meta-analysis, adjusting for clustering of information at study level. Using individual participant data over aggregate data for meta-analysis has a number of benefits including more consistent control of confounders, ability to apply the same or similar exclusion criteria and statistical analysis, and facilitation of assessments of clinically important sources of heterogeneity (i.e. effect measure modification).

In the context of research on Ebola viral RNA persistence and infectivity, IPD-MA can be used to create a larger sample size for more precise and accurate estimates of viral durations. By pooling data across studies, IPD-MA can improve the precision and accuracy of risk estimates, especially within important subgroups such as survivors co-infected with HIV. IPD-MA can also be used to partition heterogeneity into study- and participant-level, providing more statistical power to identify factors associated with long-term viral persistence. It can also be used to generate consistent analysis and confounder adjustment, facilitating inferences across studies to inform further research, surveillance and clinical interventions.

3.2 Modelling sexual transmission in a post-epidemic Ebola setting

Anna Thorson, World Health Organization

With the 2014-16 Ebola outbreak in West Africa generating the largest community of Ebola virus disease survivors in history, the issue of potential sexual transmission in a post-epidemic Ebola setting was of growing importance. Sexual transmission of Ebola virus by survivors has been demonstrated in Liberia and Guinea, where sequencing evidence indicates transmission in two separate cases 179 and 470 days after disease onset. Further evidence on sexual transmission is limited, and estimates of the risk of sexual transmission of Ebola virus disease over time will require complex research.

In an initial attempt to investigate this issue, modelling has been employed using data from an observational cohort study in Sierra Leone and WHO situation reports to estimate the magnitude and contribution of sexual transmission, and probability of an event of sexual transmission occurring after an outbreak. More work is needed to strengthen this model, however these initial findings point towards the importance of developing public health recommendations to prevent sexual transmission of Ebola virus in post-epidemic settings.

3.3 Discussion

Communication: Communicating uncertainty and risk is difficult, but must be done accurately in order to interact with the survivor community. There is an ethical responsibility to be clear about what we know and what we don't know and to give clear guidance to survivors and policy-makers.

Infection and transmission: The source of the most recent Ebola outbreak (Democratic Republic of Congo, Likati, May 2017) is unknown but may be linked to bush meat. Towards the end of an Ebola epidemic, a survivor may be the most probable source of new infection rather than a zoonotic jump.

Instances of sexual transmission of Ebola virus have occurred some time after discharge. A delay between discharge and potential transmission may not be a characteristic of the disease, but could be due to personal energy, stigmatization issues etc.

Meta-analysis: Theoretically an excellent idea but will get difficult to implement as in each study there are different periods, populations, epidemic patterns, assays etc. Is there enough data to control for all these issues? Multiple IPD-MAs could be conducted, using studies with similar data.

It is important to understand not everything is merged in a meta-analysis. We need to be clear on what we want to get out of the meta-analysis and then select the variables.

Pregnancy rates: There have been observed increases in pregnancy rates amongst female survivors reported in the PREVAIL cohort. Women report increased emotional and social desires to have children after surviving Ebola virus disease.

Semen: Semen is composed of four types of fluid – is it difficult to say what the contribution of each may be to infectivity.

Survivors who have difficulty producing samples report that this has happened after infection/survival.

Survivor stigmatization: What is the benefit for the survivors to be identified? Many survivors in Sierra Leone do not want to participate in persistence studies because they will be identified again, and may be stigmatized. Recommendations to strictly preserve confidentiality are needed.

Clinical implications of viral persistence: Are any of the symptoms considered part of the Post-Ebola Syndrome related to viral persistence? Additional data are needed.

Replication vs. elimination of Ebola viral RNA: Recent studies indicate that the RNA in semen is positive sense RNA indicating ongoing replication. What triggers or enables replication and where this is taking place is not known.

Implications for future Ebola therapeutics: Given documented sexual transmission of the Ebola virus, high EVD mortality, and high level of persistence during at least the first 9 months following EVD, the development of target product profile for EVD therapies should include these aspects.

4. Findings from national semen testing programmes

4.1 Men's Health Screening Programme, Liberia

Emerson Rogers, Ministry of Health and Social Welfare, Liberia

The Men's Health Screening Programme of Liberia was launched on 7 July 2015. Service sites are in areas most affected during the Ebola outbreak, and are located within general hospitals so presenting survivors are not specifically identified with Ebola follow-up. Mobile services are also offered. An Ebola treatment unit (ETU) discharge certificate was initially required for enrolment. As of July 2016, males self-reporting as survivors were also allowed to enrol in recognition that many survivors did not seek or receive treatment in an ETU during the outbreak. Participants are provided with counselling on safe sex practices, free condoms and instructions for use, and HIV counselling and testing if consent is given. Financial reimbursement is provided for transportation and providing semen samples. Survivors providing samples receive counselling and return in two weeks for the result. If RNA is not detected, the survivor is asked to produce a second sample and to return in another two weeks. If RNA is not detected a second consecutive time, the survivor 'graduates' from the Programme. Survivors whose tests are indeterminate or detect RNA are requested to return for testing every two weeks until RNA is undetected in two consecutive tests. Questionnaires are also administered at each visit to assess survivor adherence to and knowledge of safer sex practices.

Samples are transported once a week to the national laboratory for testing. In July 2016 the diagnostic platform was changed from the US CDC assay to GeneXpert, due to comparable results, shorter training times for technicians and consistency with neighbouring countries also using GeneXpert. With the expansion of enrolment beyond those with ETU discharge certificates, the Programme has recruited more male survivors than are registered by the Ministry of Health. Most survivors clear RNA from their semen within one year of recovery. Age seems to be a factor, with older survivors producing outlying results of 958 days (54 years) and 719 days (55 years). It is important to engage the survivor community in developing follow-up programmes. Semen testing should be implemented as soon as possible during an outbreak and should be part of a holistic approach to survivor care.

4.2 Active case finding around survivors (SA-Ceint), Guinea

Sakoba Keita, Agence Nationale de Sécurité Sanitaire, Guinea

In March 2016 Ebola virus disease re-emerged in Guinea, three months after the country was declared free of Ebola transmission. The persistence of the virus in semen and fear of repeated re-emergence has led to the development of SA-Ceint – a community-based surveillance system centred on survivors and their relatives. The programme includes surveillance, community participation through village platforms, body fluid testing, hygiene promotion, training and provision of health care to survivors and families and incentives including nutrition, financial support and community infrastructure.

The programme has established 767 functional focal points and 377 village platforms. 1128 survivors have been identified, including 375 men who have

provided semen samples. Of these men, six produced semen in which Ebola RNA was detected. Three of the men were given treatment with favipiravir, and after three weeks RNA was undetected in their semen. Potential strategies to reduce the risk of sexual transmission include treatment with favipiravir and vaccination (with the experimental Ebola vaccine rVSV-ZEBOV) of contacts of survivors who have been released from ETUs for less than twelve months. The community infrastructure projects (boreholes) provided by the programme has helped to engage communities and improve the living standards for survivors. Community involvement in SA-Ceint has supported implementation of the programme and has reduced stigma and facilitated the reintegration of survivors.

4.3 CPES – Project Shield

Kwame O'Neill, Ministry of Health and Sanitation, Sierra Leone

The Comprehensive Programme of Services for Ebola virus disease Survivors (CPES) – was established to provide the estimated 5,116 survivors of Ebola virus disease in Sierra Leone with free follow-up health care and support. Survivors can be ostracized by their families and communities, and there is much confusion regarding their health status and infectivity. Strategies to reduce the risk of sexual transmission of Ebola virus include semen testing for survivors, counselling on safer sexual practices and condom distribution. Importantly, semen testing must be embedded in counselling (community, household and individual) and safer sexual practices to create an effective strategy to reduce the risk of sexual transmission.

Project Shield is a specialized programme for male survivors aged over 15 years. Counselling pre- and post-testing addresses uncertainty on the persistence and infectivity of the virus in semen. The results of the semen test (RT-PCR to detect Ebola RNA) are reported as detected/not detected rather than positive/negative to address misperceptions that the test is testing for infectivity. As of March 2017 the Project has tested 573 survivors, with 28 showing detectable RNA in their semen and some demonstrating very intermittent detection. To avoid creating a false sense of security, 'discharge' certificates (requested by participants) were not issued – a decision that was validated after one patient who had 'graduated' from CPES later produced a sample with detectable RNA in Project Shield. Generally low viral loads have been detected, but are accompanied by high anxiety. Developing a communications strategy for the survivors and the public is very challenging, as well as sustainability of the Project after the end of funding.

4.4 Synthesis of data

Ian Crozier, Consultant, World Health Organization

The data presented during this meeting show generally homogeneous results, but there are a small number of significant outliers with longer-term seminal Ebola viral RNA detection. A wide range of questions needs to be answered about Ebola viral RNA, most importantly what the prevalence, intensity, pattern, dynamics of clearance, and maximum duration of Ebola virus RNA detection in semen is. Theoretical associations may include host characteristics, acute Ebola virus disease characteristics, or Ebola virus disease survivor characteristics. A synthesis of data from research cohorts, national programme data and other data sources raises important research questions that need to be addressed:

- What are the prevalence, intensity, pattern, dynamics of clearance, and maximum duration of Ebola virus RNA detection in semen?
- What factors are associated with Ebola virus RNA persistence in survivors?
- What factors contribute to intermittent detection of Ebola viral RNA in a given individual?
- What is factors influence infectivity associated with Ebola virus RNA persistence in semen?
- What are the risks for and determinants of sexual transmission from contact with the semen of male Ebola virus disease survivors?
- What is the correspondence between Ebola virus persistence in semen and clinical sequelae in survivors?
- What is the prevalence and risk of transmission of Ebola viral persistence in other body fluids of survivors?
- What are the optimal RT-PCR testing strategies (and pre-test characterization) to detect Ebola virus RNA in the semen?
- What is the role for antiviral or other interventions to clear or reduce Ebola virus persistence in semen?

4.5 Discussion

Boys and adolescents: Boys and male adolescent survivors are not included in semen testing programmes. Should they be enrolled when they enter reproductive/sexually active ages? Some testing programmes set this age from 18 years and above – is this realistic?

Fatherhood: Some survivors have sought assurance that it is safe for them to start families. Recommending safer sexual practices effectively instructs survivors not to have children – this is unacceptable to many people and communities. How can these messages be communicated to survivors? There are a small number of outliers who have shed RNA for extended periods of time. But should this prevent all survivors from having families?

Mental health: Continuous testing also impacts on the wellbeing and mental health of survivors.

Meta-analysis: All of the data sets are very messy and there is a lot of missing data. However with data cleaning and synthesis questions for each cohort, there is likely enough data and power in the evidence to draw meaningful conclusions.

5. Testing and assays

5.1 Semen laboratory testing: Lessons Learned

Ute Ströher, United States Centers for Disease Control and Prevention

Seminal fluid unlike other clinical specimens contains high concentrations of RNases and proteolytic enzymes. Differences between semen samples of different individuals have been observed in regard to RNA integrity. Potential contamination of semen specimens with hand sanitizer or lubricant in addition to diluting the specimen may affect the integrity of viral or cellular membranes, thereby making RNA accessible to degradation. The importance of selecting an appropriate housekeeping gene to avoid false negative Ebola virus qRT-PCR results was stressed.

The standards of semen sample collection, storage and RNA extraction can affect test performance and interpretation, however, there is no data suggesting an advantage of one Ebola qRT-PCR assay over another. The majority of published limits of detection (LOD) of different Ebola assays are given in infectious units (plaque forming unit (PFU)/ml or 50% tissue culture infective dose (TCID₅₀)/ml) making comparison difficult. Only if/when the same virus stock is used for the determination of the LOD is a relative comparison of performance acceptable.

More research is needed to investigate the persistence of Ebola virus in the male genital tract. Ebola virus qRT-PCR data of the Sierra Leone Ebola Virus persistence study indicate that Ebola virus Ct values increase over time and that there is no evidence of intermittent shedding of the virus. Alternation between positive and negative PCR results (Ebola virus RNA detected and not detected) in consecutive semen specimens are in the high Ct range and consistent with the Ct pattern observed close to the limit of detection.

The potential of a specimen containing infectious virus can't be answered by qRT-PCR; low Ct values might indicate the potential of infectious virus but other factors like humoral immune response also need to be taken into consideration.

5.2 Assay optimization and standardization for detection of Ebola virus RNA in semen

Jaime Pettitt, National Institutes of Health, USA

Various assays were initially tested for use with semen. Part of establishing the assay is understanding how the assay performs. Running panels and looking for false positives will help to select cycling conditions and eventually a realistic cycle threshold (Ct) cut-off. During the outbreak, a push was made to transition to the GeneXpert system. The cartridge-based system was quickly found to be faster and easier to use, reducing staff training time as well as contamination and safety risks. Controls are built into each cartridge allowing the system to be easily used and deployed. The platform is also familiar with public health workers in the region, being widely used for HIV and tuberculosis testing. Whole blood and semen were compared in biosafety level 4 (BSL4) laboratories, with no statistical difference noted. No false positives were detected in the laboratory deployed in the field. However it is important to remember that a test is a 'snapshot' of a sample, if something isn't detected, it does not mean that it is not there.

Experiments on the effect of temperature on sample degradation indicate that keeping samples frozen seems to be the best way to conserve an accurate Ct value. If testing cannot be done on the same day as sample collection, freezing is the best way to preservation method. The need to set Ct thresholds for different assays raises issues of subjectivity and comparability. The GeneXpert test produces an automated reading and therefore avoids variability in interpretation. One option to compare test is to develop a standardized panel for assay comparison. Each test has its uses and should be chosen to best fit the laboratory.

5.3 Discussion

Assay cartridges: Can WHO play a role in regulating access and cost of GeneXpert assay cartridges? Currently the only prequalified assay cartridges are for HIV, tuberculosis (TB) and human papillomavirus (HPV). In the field of TB, most patients are also co-infected with HIV. Most times samples are taken, but there is a shortage of cartridges. Unitaid could be engaged to help negotiate with the manufacturers, and have done so for HIV and HPV.

Diagnostics: The right test for the right time is needed. More work is needed to standardize tests and to have global stocks of assay cartridges for testing.

Sample ownership and future use: There are also questions of ownership of samples by the country and by the patient. The samples are a special and important scientific resource and the next generation of local researchers should be encouraged to reanalyse them. Countries should decide on their own priorities for this resource and should hold discussions with partners on mutually beneficial agreements for biosecurity and storage.

Sample storage and preservation: Operational guidance for sample storage, transport, preservation and biosecurity should be reviewed. Variability in these issues can effect integrity of RNA and subsequently detection.

6. Revision of public health recommendations

6.1 Revision of current WHO interim advice on sexual transmission of Ebola virus disease

Pierre Formenty, World Health Organization

The current WHO interim advice on sexual transmission of Ebola virus disease was updated on 21 January 2016. The document provides an introduction to the body of knowledge on the issue and seven interim recommendations based on this evidence. The interim advice needs to be updated in light of the research presented during this meeting and to reflect new epidemiological and scientific findings. It was recognized that in developing public health recommendations, a balanced judgment is required between the quality of evidence, benefits and harms. Costs, values and preferences also need to be considered when developing practical recommendations.

6.2 Discussion: revision and update of recommendations

General

- These recommendations have been developed to guide national health programmes in affected countries. Countries should make a decision to implement them based on their own national contexts. They are public health recommendations, not individual patient recommendations, and should be adapted by national programmes based on their own context or epidemiology.
- The effectiveness of latex condoms should be reviewed to explain why condom use is recommended. There are currently no studies that have demonstrated the effectiveness of condoms to prevent Ebola virus transmission.
- The issue of persistence of RNA in semen should be differentiated from infectivity in the advice.

Introduction and summary of evidence

- An additional three events of sexual transmission of Ebola virus have been reported in the literature, and should be added to the one reported event described in the current advice. The exact mode of infection in these events still cannot be clearly identified.
- The length of time for which Ebola virus and RNA can be respectively isolated and detected from semen after symptom onset should be updated with new data, for example: evidence of live Ebola virus has been reported after 233 days post onset of symptoms in the BNI cohort, Guinea (SCID mice injection) and virus isolation was described 157 days in the SLEVPS cohort, Sierra Leone (Vero cell culture); and maximum length of time for RNA detection has been reported after 965 days in the ELWA Cohort, Liberia by Fischer et al. in "Ebola Virus RNA Detection in Semen More than Two Years After Resolution of Acute Ebola Virus Infection" *Open Forum Infection Diseases*, 2017.

- Comments on isolation of Ebola virus and RNA from vaginal fluids should be updated with current data. Ebola virus RNA has been detected by RT-PCR in vaginal fluid from one woman 35 days after symptom onset (Sierra Leone, 2016). Of note, several cohort studies have generated a large number of negative vaginal fluid results, the vast majority of which have been collected six months or more after discharge from an ETU.
- There is a critical responsibility for WHO to provide data and evidence with the guidelines so that countries are fully informed of any associated risks.

Recommendation 1

- Some participants feel that the term 'ETU' should be removed from the advice in order to normalise the language around Ebola. Other terms such as 'isolation unit' are also not acceptable in some communities. The term may be replaced by 'Ebola care and treatment centre' or similar.

Recommendation 2: Date of enrolling survivors in semen testing programmes

- The diagnostic utility of the first recommended semen test within three months after onset of disease is debatable. The result of this test is likely to be positive, however some survivors are negative before this period and would benefit from earlier testing. This may be however costly and would add little from the public health perspective.
- In contrast, conducting the first semen testing at discharge was proposed as a way to engage male survivors in semen testing programmes as soon as possible. However, it is mentally and physically difficult for survivors to give samples at this stage. At discharge the survivor has important personal concerns around family, fear and stigmatization to address. They first need counselling and time to mentally recover.
- In summary, male Ebola survivors should be enrolled in semen testing programmes when discharged starting with counselling and distribution of condoms, and offered semen testing when mentally and physically ready, at three months maximum after disease onset.

Recommendation 3: Testing to stop after two undetected (negative) results

- Current advice recommends that testing can stop after two consecutive results are obtained where Ebola RNA is undetected (i.e. negative result) in semen, at least two weeks apart. However there have been a small number of cases of intermittent detection where Ebola RNA has been detected (i.e. positive result) after these two consecutive negative results have been obtained. For these survivors with intermittent detection of Ebola RNA in their semen, the test results were very close to the limit of detection of the test (RT-PCR) which may indicate an extremely low risk of infectivity. Of note, there have been no reported cases of sexual transmission linked to these survivors with intermittent detection of Ebola RNA in their semen.
- One option may be to recommend that survivors be tested annually after two undetected (negative) test results. This strategy could create high ongoing costs and should be balanced with the public health benefits.

- The phrase “without fear of transmission” should be replaced with “with minimized risk” to reflect the science more accurately.
- In conclusion, it was agreed that after having obtained an undetected (negative) test result twice as described above, survivors can safely resume normal sexual practices with reduced risk of Ebola transmission.

Recommendation 4: 12-month period for safer sexual practices

- All survivor cohort studies and national semen testing programmes discussed during the meeting have identified survivors who have produced RNA fragments in their semen after more than one year. Some participants voiced the opinion that the recommendation for abstinence or practice of safer sex should be extended from 12 months to the maximum result (965 days, or approximately 32 months).
- It was noted that this recommendation is for exceptional circumstances where a survivor is not able to access testing. In these circumstances, a more conservative period may be considered.
- Other participants did not believe that the current period should be extended. On a population-based level, these outlying cases do not have significant impact. It may be unwarranted to base a recommendation for all survivors on a small number of outlying results, particularly given the lack of evidence around infectivity.
- Many survivors are anxious to resume normal sexual activity and to start families. A recommendation to delay this further has major impact on their emotional and social wellbeing. Recommendation for a longer period for abstinence or safer sexual practices would also be very difficult for the affected countries to implement.
- There was little support for decreasing the period.
- Adding a qualifier of ‘minimized’ risk to a recommendation of the existing 12-month period could be a compromise.

Recommendation 5, 6 and 7: no change

Follow-up testing, counselling and treatment

- It is important to start follow-up/engagement with survivors as soon as possible after treatment for counselling as well as long-term care and testing. Losing survivors to follow-up is a major issue and more work needs to be done to better engage and retain survivors.
- There should be a clear recommendation for survivors with detected (positive) results on how to access treatment. However, at present no treatment has been validated.
- Semen testing should also be integrated into a wider recovery and follow-up plan including other guidance and tests (e.g. ophthalmology).
- There is an issue of compliance with condom use. Sexual health programmes could advise on how to improve adherence to safer sexual practice.
- In the long-term, integration should be considered into other health programmes, such as HIV with decentralized testing centres and

voluntary testing. This should also be expanded beyond testing to include advice, support and counselling for survivors.

- Most adolescent male survivors will age into the cohorts that are being tested. They should be provided with safer sex messaging to prevent potential Ebola transmission as well for general sexual health. This could be used as an opportunity to strengthen school health education programmes.
- Spouses/sexual partners should also be included in recommendations on counselling.

Feedback from survivors

- Survivors have requested clearer recommendations that are more promoted and publicized. They have also requested research and advice for couples who wish to proceed with pregnancy.
- Survivor organizations commented that it is very difficult to determine what and how to communicate to survivors, given the uncertain evidence base. The lack of clarity is very frustrating for survivors.

Language and terminology

- The term 'safer' sexual practice is used in recognition that these practices offer, but do not guarantee, protection from sexually transmitted infections (STIs). This language is used by UNAIDS and other international organizations specializing in sexual and reproductive health.
- The date of "onset of disease" and "onset of symptoms" are used as a baseline from which to time testing and abstinence/safer sexual practices periods. This date can be hard for survivors to remember or estimate. It may be more practical to change this to the date of ETU discharge or date of recovery.
- Where relevant, language should be used that is consistent with HIV programmes (e.g. when describing couples with discordant HIV status).

7. How to get better prepared for research?

Major issues discussed in this session are summarized below:

Community engagement: More emphasis is needed on communication, community engagement, and social mobilization. Social and anthropological issues are not currently addressed adequately enough in work around viral persistence. Community engagement was pivotal in stopping the Ebola outbreak, and will also be needed to socialize messages around the Ebola vaccine.

Diagnostics: There are many tools available and ideas for new methods. This is now a good time to take an inventory of these diagnostics methods to see how research can be best pursued and treatments determined.

Loss to follow-up: Losing survivors to treatment and research follow-up is an important issue. Governments could take the lead in working with research institutions to encourage survivors to continue participation in programmes.

Public health: Public health and research are complimentary, but the gap between research and public health needs to be bridged. Strong public health systems with good surveillance are needed to help to define what the research questions should be. The translation of evidence into policy and public health action also needs to be strengthened, and could be done in partnership with public health institutions and international partners.

Research capacity development: Research infrastructure needs to be built and sustainable research capacities strengthened. A lot of capacity and infrastructure has been lost in the tertiary sectors of the affected countries. Research capacity and training should be strengthened at universities, Ministries of Health and public health institutes, to develop knowledge and skills. We need to encourage and foster young scientists to be prepared for the next emergency.

Research collaboration: Regional research cooperation through common protocols and standardization between outbreaks will enable research collaboration during emergencies. There is an opportunity to develop systematic mechanisms for the three countries to work on research together and pool resources. Conditions can be created to allow more flexibility to implement studies across borders, utilizing for instance the Sub-Regional West African Consortium for Clinical Research or a designated research authority body.

Research funding: Research was able to start quickly during the outbreak, but could have moved faster if funds could have been mobilized more quickly.

Research tools and protocols: New tools have been developed that were not available at the start of the outbreak, including databases and generic research protocols that can be adapted to other countries and/or diseases when an outbreak occurs. Other protocols can be developed in advance for issues such as multiplex PCR for viral haemorrhagic fevers and other clinical syndromes; virologic persistence studies embedded into long term follow-up

of survivors; standardization and use of most sensitive assays; common testing frequencies; and community advisory boards for target populations.

Treatment: Not enough is known about the effectiveness of medical countermeasures use to treat Ebola disease and to clear Ebola virus from privileged sites. Immunization is another area that will need more future attention, with the ongoing development of Ebola vaccine. Complications in survivors also need to be carefully studied, and efforts made to distinguish health effects due to Ebola versus other causes.

WHO Blueprint: WHO has set out its strategy for research and development in the *R&D Blueprint* (<http://www.who.int/blueprint/en/>). The Blueprint has identified an initial list of priority diseases for research and development, including Lassa fever, filoviruses diseases (such as Ebola and Marburg) and Rift valley fever. Diseases identified in the Blueprint should be made national priorities if endemic in a country, and national researchers should be at the forefront of this work.

Women's health: Issues around Ebola and women's health such as pregnancy have not been strongly addressed. The PREVAIL birth cohort study is making some efforts towards addressing this, but is limited.

8. Work group discussions on research gaps

Participants chose to join one of four groups to discuss research gaps in areas previously identified in Session 4 of the meeting (see Annex 2: Agenda). Each group was asked to discuss ideas for activities and implementation for their area, and if possible to identify a timeline, funding and remaining research questions.

8.1 IPD-MA

Facilitator: Nathalie Broutet, World Health Organization

There was consensus on the principles of meta-analysis and the benefits that it could bring to our understanding of viral persistence in semen. Individual studies are not powered to assess clinically important sources of heterogeneity. IPD-MA offers opportunities to understand the correlation between ongoing symptoms and viral persistence and to better assess the validity of rare phenotypes. The level of collaboration and data cleaning needed for IPD-MA will also inform the development of standardized protocols.

The main objectives for an IPD-MA would include applying consistent and confounder analysis to measure the duration of viral persistence in semen across studies; leveraging data from all studies to identify and quantify sources of heterogeneity; reaching a better understanding of intermittent detection versus intermittent shedding; and understanding ongoing sequelae in survivors. A decision to participate in an IPD-MA should involve investigators, Ministries of Health and other stakeholders. A data sharing agreement will need to be developed to protect the intellectual property of individual studies and participant confidentiality. Ethical concerns for surveillance versus research data also need to be addressed.

The group proposed to convene working group to develop and share a concept note clarifying the objectives and utility of IPD-MA. Further research questions in this area included: risk factors for positivity in semen; factors for Ebola virus disease survival; and mortality and survivors.

Summary of key research questions

Sexual transmission

- What are the factors influencing sexual transmission?
- What is the likelihood of new infection due to sexual transmission, after an epidemic has ended?

Persistence

- What is the probability of detecting Ebola virus RNA in the semen of survivors after 12 months?
- What are the factors responsible for clearing the virus from the MGT (humoral/cellular immune response)
- What is the duration of persistence of Ebola virus in semen?
- What factors influence seminal Ebola viral RNA persistence in semen?
- What factors are responsible for intermittent detection of Ebola viral RNA in semen?

8.2 Laboratory

Facilitator: Pierre Formenty, World Health Organization

Further investigations are required to determine the infectivity of semen specimens. These may include evaluation of additional cell lines (e.g. HuH), stripping antibodies from the virus and retrying virus isolation, further work with SCID mouse models, and identifying factors that determine systemic vs. localized infection.

The term 'intermittent detection' should be used to describe PCR results instead of 'intermittent shedding'. 'Shedding' should only be used when there is evidence of infectious virus production. Specific language should also be used when describing positive PCR results – a positive result indicates the detection of Ebola RNA, not necessarily infectious live Ebola virus. Housekeeping genes used in different assays should be compared. PCR results should be reviewed to assess if intermittent detection is only observed at the limit of detection of the PCR assay used or if it is indicative of intermittent virus shedding. It is also important to review the evidence behind the recommended number of Ebola RNA negative tests (two) and the time interval after which specimens should be collected (two weeks) in the current WHO interim advice.

The group discussed activities to standardize semen samples and testing. Development and dissemination of a document to provide guidance on sample collection, transport, storage and testing timeframe is required. Specimens should be collected without lubricant or hand sanitizer, refrigerated after 1 hour and tested within 72 hours of collection. There is no need to limit testing to a particular PCR test or platform, but housekeeping gene controls must be run on each sample to avoid false negative results. Based on existing data, the sensitivity of GeneXpert, Altona & CDC Ebola assays are sufficiently comparable to be included in a meta-analysis.

Summary of key research questions

Viral shedding

- How can intermittent shedding vs. intermittent detection be determined?
- Is there intermittent replication from a persistent viral reservoir?
- How long does the virus persist in semen?
- How many negative PCR test for viral RNA are needed to rule out seminal viral persistence?
- What is the longest time interval between seminal viral detection?

Infectivity

- What is a definition for infectivity?
- How can infectivity be tested for?
- Can a proxy or surrogate for infectivity be validated/used?

Semen

- How should semen be collected to avoid contamination?
- How should semen be transported and stored to avoid damage?
- How should semen be prepared before testing?
- What PCR controls should be used and how should they be interpreted?

- How can the interpretation of results from different PCR platforms/tests be standardized?
- What is the gold standard diagnostic testing for semen screening and detection?
- Is there adequate positive predictive value in the post EVD setting to accept one Ebola gene target as a detection?

8.3 Generic protocols

Facilitator: Emerson Rogers, Ministry of Health and Social Welfare, Liberia

Generic protocols can be developed for a viral persistence study by drawing on existing protocols from Guinea, Liberia and Sierra Leone as well as protocols developed for Zika virus research³. Generic protocols could also be developed for clinical trials (e.g. PREVAIL IV), population sero-prevalence studies and for the long-term follow-up of survivor and close contact cohorts over a suggested period of 5-10 years.

Long-term follow-up may pull resources from already weak health systems. To mitigate this effect, testing centres could be decentralized using the GeneXpert platform and mobile-health initiatives. It is important to maintain government ownership over survivor programmes, which could eventually be integrated into other elements of the health care system for better sustainability. Future needs will include training counsellors for long-term follow-up, and providing communication platforms for survivors to be contacted for appointment reminders and to report associated symptoms. Finding resources to fund this area of work was noted as a major challenge.

Summary of key research questions

Treatment and protocols

- Are drugs (e.g. favipiravir, Gilead) effective in clearing the virus from the semen reservoir?
- What is the target product profile for therapeutic candidates to reduce viral persistence and eradicate virus from the semen?
- Preparation of a clinical trial with generic protocols to evaluate:
 - Favipiravir
 - Gilead (compound GS-5734)
 - Placebo

8.4 Messaging / advocacy to survivors

Facilitator: Axelle Ronsse, Médecins Sans Frontières

The main concerns and questions from the survivor community include:

- When is the virus gone completely from the body?
- What does “detected” actually mean?
- What can be done (e.g. medicine, behaviour) to get rid of the virus faster?

Messaging to survivors should communicate that the answers to this questions are not completely known yet, but research is ongoing and results

³ See *Standardized Research Protocols* available at <http://www.who.int/reproductivehealth/zika/zika-virus-research-agenda/en/> (accessed 1 September 2017).

will be shared with the communities as they are learned. It is important to emphasize that we do know that the risk of transmission of Ebola virus from survivors is low, and survivors should be encouraged to continue to participate in programmes and research while we work to learn more.

Male survivors should be recommended to continue testing until Ebola RNA is undetected in two consecutive semen samples. While being followed-up for testing, they should practice safer sex using condoms. Counselling should be provided to male survivors undergoing testing, as well as for partners concerned about resuming normal sexual practices or starting a family.

A one-page document to communicate key messages and (updated) WHO recommendations should be disseminated to all survivor organizations. It is essential to maintain clear, accurate and regular communication with survivor communities, governments and other partners.

9. Next steps

The following next steps were agreed on by participants to advance the work discussed during the meeting.

9.1 Revise WHO interim advice

The current WHO interim advice on sexual transmission of Ebola virus disease should be revised based on the comments and discussions held during this meeting. *Lead: WHO.*

9.2 Develop concept note for an IPD-MA

The meeting agreed that there is added value in pursuing an IPD-MA using data from existing and ongoing studies on Ebola virus disease and survivorship. To move this forward, an IPD-MA concept note will be developed to clarify objectives and address concerns on intellectual property, patient confidentiality and ethics. A proposed working group will take on this responsibility. *Lead: WHO.*

9.3 Standardise protocols for semen collection and storage

Participants noted a wide range of differences between studies and public health programmes in how semen and other samples from Ebola virus disease survivors are collected and stored before testing. In order to improve the comparability of data, these processes should be standardised. A document providing guidance on standardised protocols for semen collection, transport, storage and testing will be developed and disseminated. *Leads: WHO, NIH, US CDC.*

9.4 Development and continuation of research protocols

The meeting agreed on the usefulness of generic research protocols for studies on 1) clinical trials of substances to reduce Ebola virus persistence in semen; 2) Continued long-term follow up of cohorts; and 3) sero-prevalence of Ebola virus disease in populations 4) Clinical implications of Ebola viral persistence. The development and implementation of these protocols will require ongoing discussion. *Leads: WAC, WHO, NIH, CDC.*

9.5 Communication and advocacy document

The importance of communicating recommendations and scientific knowledge clearly and accurately to survivors, their families and communities was stressed throughout the meeting. Clear messages must be developed to communicate the updated WHO interim advice and to encourage survivors to participate in support programmes and research. A short (e.g. one-page) document will be developed to communicate key messages and updated recommendations and disseminated to country survivor organizations, governments and other partners. *Leads: WHO, John Snow Incorporation, PREVAIL-NIH.*

Table 1. Summary of next steps

Items	Lead institutions	Activities	Timeline
1. Revision of WHO interim advice on Ebola sexual transmission	WHO	<ul style="list-style-type: none"> • Development of systematic reviews • Virtual meeting of the Guideline Development group • Submission to the WHO Guideline Review Committee 	31 Oct 2017
2. Concept note for an IPD-MA	WHO	<ul style="list-style-type: none"> • Facilitation of working group • Preparation of concept note for publication 	15 Oct 2017
3. Protocols for specimen collection and storage	CDC, WHO, NIH	<ul style="list-style-type: none"> • Review of existing protocols • Draft standardized protocols • Review by partners 	31 Oct 2017
4. Generic research protocols	WAC, WHO, NIH, CDC	<ul style="list-style-type: none"> • Review of existing protocols • Facilitation of partners discussion • Draft generic protocols 	End 2017
5. Communication and advocacy document	JSI, WHO, PREVAIL-NIH	<ul style="list-style-type: none"> • Draft advocacy document summarizing state of knowledge • In-country dissemination 	Oct 2017

10. Closing session

On behalf of the WHO Representative, Dr Zachari Wambaai commended the participants for their active participation during the meeting. Many expectations have been created, and we need to continue these important activities beyond these discussions. Concrete actions are already being developed and should be acted on quickly. It is especially important to develop communication messages on this work to be able to speak clearly to Ebola virus disease survivors and their partners.

Dr Pierre Formenty thanked the participants and representatives from Guinea, Sierra Leone and the host country Liberia. He also thanked the researchers who had participated in the meeting for the quality of their presentations and for sharing their data with transparency and honesty. The work needs to continue, and could expand to other countries that have been affected by Ebola outbreaks such as the Democratic Republic of Congo and Uganda. The participants to this meeting represent a large community of researchers and public health professionals engaged in this issue, making sure that the survivors of the Ebola outbreak are not forgotten. It is hoped to expand this collaboration to other issues such as clinical management, to improve our knowledge and the care we can offer to people affected by Ebola virus disease.

Annex 1

List of participants

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Annex 2

Agenda

Wednesday, 28 June 2017		
Time	Session	Topics
8:45 – 9:00	Session 1: Opening	<ul style="list-style-type: none"> • Opening remarks • Welcome address • Meeting objectives
9:15 – 10:45	Session 2: Findings from cohort studies	<ul style="list-style-type: none"> • PREVAIL III natural history study, Liberia • PostEboGui cohort study, Guinea • Sierra Leone viral persistence study, Sierra Leone
10:45 – 11:15	Coffee break	
11:15 – 12:45	Session 2: (cont.)	<ul style="list-style-type: none"> • UNC-ELWA-CRM cohort study, Liberia • BNI-EU-INSERM cohort study, Guinea • Ebola survivor follow-up, Guinea
12:45 – 14:00	Lunch	
14:00 – 15:30	Session 3: Further learning from cohort studies	<ul style="list-style-type: none"> • Using IPD-MA to inform public health recommendations for emerging pathogens • Modelling sexual transmission in a post-epidemic Ebola setting
15:30 – 16:00	Coffee break	
16:00 – 17:00	Session 4: Research gaps	<ul style="list-style-type: none"> • Study of the immune response, significance and impact on the natural history, and protection • How and where does the Ebola virus persist in the body of survivors? • Implications of viral persistence for treatment trials and follow up guidelines
17:00 – 17:30	Wrap up	

Thursday, 29 June 2017

Time	Session	Topics
9:00 – 10:30	Session 5: Findings from national semen testing programmes	<ul style="list-style-type: none">• Men's Health Screening Programme, Liberia• Active case finding around survivors (SA-Ceint), Guinea• CPES – Project Shield• Synthesis of data
10:30 – 11:00	Coffee break	
11:00 – 12:30	Session 6: Testing and assays	<ul style="list-style-type: none">• Semen laboratory testing: Lessons Learned• Assay optimization and standardization for detection of Ebola virus RNA
12:30 – 13:30	Lunch	
13:30 – 15:30	Session 7: Revision of public health recommendations	<ul style="list-style-type: none">• Revision of current WHO recommendations on sexual transmission of Ebola virus disease
15:30 – 15:45	Coffee break	
15:45 – 17:00	Session 8: How to get better prepared for research?	<ul style="list-style-type: none">• Facilitated discussion
17:00 – 17:30	Wrap up	

Friday, 30 June 2017

Time	Session	Topics
7:45 – 9:15	Field visit	<ul style="list-style-type: none">• Optional field visit to JFK Hospital, Monrovia
9:30 – 11:00	Session 9: Work group discussions	<ul style="list-style-type: none">• IPD-MA• Laboratory• Generic protocols• Messaging / advocacy to survivors
11:00 – 11:30	Coffee break	
11:30 – 12:30	Session 9: (cont.)	<ul style="list-style-type: none">• Feedback
12:30 – 13:00	Session 10: Closing	<ul style="list-style-type: none">• Wrap up• Closing remarks