CEPI | New vaccines for a safer world

Coalition for Epidemic Preparedness Innovations

1st Biological Standards and Assays Workshop

12 December 2017

At Wellcome Trust, London

Workshop Notes for Participants

Background

New outbreaks of infectious diseases are emerging frequently, with larger transmissions, more global spread and greater economic impact than occurred historically. Despite the outbreaks of the past, we are still ill equipped for dealing with emerging outbreaks. These incidents can overwhelm the stretched capacities of many health systems. As part of the global effort to respond to these needs and build a better response, new approaches for vaccine interventions are emerging. Biological Standards are essential to enable these products to be evaluated properly, both for their own progress and to compare different vaccines.

Through the enhancement of standards, assays and animal models in this area, a better response can be mounted to these growing needs with more informed and better understood options available. This requires a collective agreement to work under common processes and collaborative strategies. Through a targeted approach, starting with CEPIs priority diseases, known methods for standards and assays can be advanced and the efforts can be made more effective.

The significance of regulatory science in an outbreak and in preparedness for outbreaks is enormous. Considerable regulatory challenges exist, especially with the pathogens CEPI is targeting. CEPI will have to face these, both in supporting advancement of our vaccine candidates and in working with regulatory colleagues worldwide.

The Coalition for Epidemic Preparedness Innovations

CEPI was established in the aftermath of the Ebola outbreak as a means to address the needs and gaps in the development of vaccines against known threats (for which insufficient development has currently occurred) and to address new emerging threats rapidly. CEPI was founded in January 2017 with the mandate to develop vaccines for a safer world, through the use of targeted funding and promotions of vaccine development where that has hitherto failed.

CEPI consists of three main operational arms:

A **Secretariat** to engage and develop work streams, subdivided into one Vaccine Science and another Vaccine Development team supported by focused working groups (for "cross cutting" issues) and external experts and partners;

The Secretariat is supported by the **Scientific Advisory Committee (SAC),** which provides scientific guidance to direct activities and advice on scientific issues.

Both of these entities work with the **Joint Coordination Group (JCG)**–the intentions of which is to align and coordinate efforts between major **CEP**I entities acting in this sector.

These three arms together form CEPI, which is building tools to develop candidate vaccines, and intervention instruments for other groups to use. The **Biological Standards and Assays working group** will initially focus on the three CEPI prioritized pathogens (as developed under the agenda of CfP1). The work of CEPI's three arms can be grouped into two areas: Response and Preparedness. CEPI activities in the first area are currently focusing on "Finishing Ebola" and the First Call for Proposals (CfP1). The second area includes the CfP2 for "Platform Technologies" and plans for other "Rapid Response activities".

The CfP1 targets three priority pathogens (Lassa, MERS-CoV, Nipah), aiming to develop vaccines from late pre-clinical or initial production, through to proof of concept in humans, in advance of an outbreak. CfP1 calls for proposals covering all steps towards "Readiness for Efficacy Studies during an outbreak" Implying GMP material manufactured for Phase II clinical trial quality and having Clinical Safety and Immunogenicity data available. This includes developing candidate vaccines through Phase I and II, performing efficacy testing where possible and potentially establishing an investigational stockpile. CfP1 is working currently (early 2018) with 14 potential vaccine products: six for Lassa and four each for Nipah and MERS-CoV. These are currently in the process of potentially being selected to become partners with CEPI through signed agreements in the first half of 2018. In early 2018, CfP1 applicants are at the Due Diligence stage and applicants are in the process of developing assays with some established already, and therefore work will be aiming for assay selection and targeted to validating assays, establishing standard reagents, appropriate selection of animal models, material sharing and SOPs. The second Call for Proposals (CfP2) is targeting platforms for rapid response and aiming for developing partner agreements by the end of 2018.

For both calls, CEPI currently operates according to the WHO Target Product Profiles (TPPs). There will be a need to keep up to date with emerging epidemiology and needs. The WHO TPPs have both ideal and minimal needs, with the former being very aspirational and the latter being rather basic. For example, the Lassa TPP asks for (ideally) vaccines against multiple Clades of Lassa but with little epidemiological or pre-clinical evidence of strain cross-protection.

CEPI has mapped the strains used in the CfP applications, which have largely, been selected by the various developers. However, despite the current stage of development there is still a need for guidance and Standard Operating Procedures (SOPs for strain selection in general and for challenge studies, reagents to use, production and for assay selection and performance) to guide early strategy choices for Lassa, MERS-CoV and Nipah. Further, there is a need to identify funding gaps, map capacities and establish understanding of needs and best practices in all areas; including for the area of Biological Standards and Assays, given the many "cross-cutting" work areas, when developing vaccines for the three diseases:

- Regulatory
- Standards
- Phase I/II capacity
- Phase III preparation, ability, design and location (also sustainability of capacities in advance of outbreaks in areas in most need)
- Data sharing (important to CEPI and well supported by applicants, except for head-to-head studies, where some reservations have been expressed)
- Routes to licensure (such as Lassa, where many potential routes need to be investigated)

The aim for the first meeting of the Biological Standards and Assays Working Group was to allow members to meet and get to know each other, build a rapport (covering the starting point and listing the essential first steps in the area) and to prepare for effective collaboration. Sharing lessons learned from Ebola and existing knowledge of the three pathogens were also core purposes for the meeting. For this work-stream it is very important to connect the different areas of CEPI and it is important to build this as a "cross-cutting" system. The aim is to plan and prepare to be pre-positioned for outbreaks, supplying material and agreeing on standards. Moreover, the goal is to bring people to "the table" and collaboratively make a more effective system, and identify areas of potential collaboration.

The meeting goals:

- Review the working group activities
- Provide an overview of potential membership
- Built Task Forces and establish where there are particular need for them
- Review the Terms of Reference
- Plan the next steps
- Propose the key routes for the work in this area in order to make substantial progress

Basics on Biological Standards and Standardization

Biological Standardization plays a major role in advancing vaccines. This can be learned from the origins of standards. Antitoxin was developed in Germany and France, but early attempts failed in the UK. The response was to develop standardised lots, which were developed under Paul Ehrlich in Germany. The first global monitor on standards was established as a committee under the League of Nations and the role or activity passed on to currently been taken care of by the Expert Committee on Biological Standardization (ECBS) in the system of the World Health Organization (WHO). International Standards enable the harmonisation and standardisation of assay data; which in turn allows evaluation of product development as well as on-going quality control throughout a product's life cycle. This monitoring facilitates progress from laboratory to clinical use. Standards require "like *versus* like" use and comparison. Moreover, standard units as the essential metrics. Biological Standards enable relative quantification of outcomes between laboratories and products.

Most standards are bulked, stabilised and freeze-dried, for long(er) shelf life and ease of distribution. All fills for a reference sample must be produced in a single batch, in a single run in one working session and they are characterized and assessed for stability. A collaborative study is then undertaken to calibrate the proposed standard and to see if it is fit for purpose. Typically, a collaborative study involves 5-25 participants representing the end users, from all global regions including higher and lower economically developed areas. Data analysis considers all aspects of the collaborate study. From this collaborative study, a project report is developed for the participants and for the WHO ECBS with recommendations for establishment and use of the preparation as and International Standard Preparation. Standards are then subsequently held at a number of locations including the FDA, NIBSC, and the Paul Ehrlich Institute.

The full process to develop and study a standard takes approximately 36 months or longer though faster routes, such as the use of interim standards or reference materials, may be available. However, issues arising may also lead to delays. The ECBS only meet once a year and this can add 12 months to the development time if the completion of the product is just after the annual ECBS meeting. Interim status standards may not have sufficient stability and may not have gone through full review process.

Regulatory Considerations for Biological Standards and Assay Development

Standards are extremely important to the regulatory process. They characterize performance evaluation, validation and other elements for comparison. At early developmental phases, this should be appropriate to qualify the assays being used, while in the later stages this is specifically measuring product critical quality attributes. The process is protocol driven and SOPs use values based upon predefined criteria; *assay criteria are defined during development and the SOP is finalized before qualification. The validation is also protocol driven and the assays are performed according to a finalized SOP*. The assays that must be validated at Phase III are those that will be used to measure clinical outcomes (primary or secondary endpoints). System suitability criteria as well as tracking and trending of assay performance over time are essential for long-term control. Qualification and validation can expedite regulatory approval, as can collaboration between Regulatory groups, joint reviews and common processes along with workshops and committee meetings with public regulatory and advisory groups.

At the nonclinical level, the use of standards and assays informs safety, advances outcomes and contributes to satisfying the regulatory requirements such as the US FDA's Animal Rule (21 CFR 314.600 through 314.650 for drugs or 21 CFR 601.90 through 601.95 for biological products). Clinical studies are easier to review through concurrency across sponsors and across studies. Establishment of standards for use during pre-clinical work (CMC-type) often lag behind the clinical work. Investing also good and early here can be of great help for the pre-clinical stage as well as for the different clinical phases and the full vaccine evaluation and potential comparison between products.

The US FDA is open to discussion at any point in the development process. The FDA has a Technical Working Group to guide development as an informal process to help advance the work, including for standards and assays. PRE-IND meetings allow a more formal mechanism for guidance and Master Files (for assays rather than standards, though t*he Master File can also include reports for the assay reagents, including the Reference Serum, Negative Control Serum, and Quality Controls*) allow regulatory review for multiple collaborators on a common product or assay through cross referencing the Master File.

FDA Animal Rule; Applied to MERS-CoV Vaccine

The FDA animal Rule is a pathway for licensure when adequate, well-controlled clinical studies in humans cannot be ethically conducted, and field efficacy studies are not feasible. Bridging is needed from non-clinical, animal immunogenicity and efficacy data to human immunogenicity

and requires standardised species-neutral assays. Important criteria for a robust animal model include a good understanding of the natural history/pathogenesis and time course of an infection in the chosen animal model. The selection of an animal model for an efficacy study should be based on its adequacy as a model of key elements of the human disease or condition and its suitability with regard to the investigational drug and how closely the animal model reflects human disease.

Standardising as many elements as possible is essential for generating reproducible and relevant data. The standardization of the challenge material is therefore particularly critical. Documentation must be thorough and complete. Variables should be controlled, wherever possible and tracked where not. All studies should be blinded to remove bias.

FDA guidance through documents and early engagement increases the likelihood of success and sponsors should be involved wherever relevant. Two FDA checklists guide this process: "Essential elements of an animal model" and "Elements of an adequate and well-controlled animal efficacy study protocol". Through the use of these documents it is possible to clearly track all needs and ensure there are no gaps in the study design. Use of checklists along with a good quality management system, with data quality and integrity throughout, are key to effective preparation for animal rule approval.

Work is ongoing to develop a suitable model for MERS-CoV. This would need a decision on the appropriate strain to use for the production of large quantities of challenge virus, standardization of the aerosol (virus) delivery method; as well as the plaque reduction assay, immune response assays, and quantitative RT-PCR for MERS-CoV.

Lessons from Ebola

In 2013 there was a new outbreak of Ebola on a hitherto unseen scale. No vaccines had been developed to licensure or even to Phase I clinical testing and no therapeutics were known. Many developers came forward in response to this outbreak.

The Filovirus Animal Non-Clinical Group (FANG) provided a framework to coordinate the many US bodies working on interventions in response to this outbreak. FANG had multiple focal areas; including "Assays, Standards and Animal Models". FANG proposed assays to be used at different stages in various ways. Systematic selection was used to identify, select and characterize strains, which was conducted SOP, with clear and detailed data capture forms. Good data documentation being key for use. Characterization was for identity, content, purity, quality and potency and from this a certificate of analysis was generated.

During outbreaks of this nature (and especially when preparing in advance of them), it is important to consider the "Animal Rule" as a pathway for licensure. This is highly relevant when adequate and well-controlled clinical studies in humans cannot be ethically conducted because the studies would involve administering a potentially lethal or disabling substance or organism; to healthy human volunteers or field efficacy studies are not feasible. This requires bridging non-clinical immunogenicity and efficacy data to human immunogenicity data, which can be used to predict likely clinical benefit in humans. In turn, this requires standardised assays. Where a correlate of protection is clearly identifiable; that would be of substantial importance. As a part

of this process, it is possible for groups to submit a Master File to the FDA for the assay, which partners or product developers can then cross-reference in their regulatory submissions. For the FANG EBOV anti-GP ELISA in the Ebola response, this process took 2.5 years from early assay development to validation and finally FDA concurrence the assay was fit for its intended use.

During the Ebola epidemic, access to sufficient reference material, such as for immunoassays, was problematic. The background-levels between African *versus* non-African samples differed substantially. This caused difficulties in developing reference materials for immunoassays (the FANG anti-GP ELISA). This process resulted in development of a 1st generation reference standard, which consisted of pooled convalescent sera from a very small number of individuals to larger pools of vaccinee sera. Subsequent to this, to even larger pools of clinical trial samples generated using a larger number of different vaccine platforms. For future consideration, some alternative options exist in cases where there are limits to obtaining sufficient volumes of human immune sera of adequately high titres; for example, purified humanised IgG generated from hyper-immunization of cows harbouring a human transchromosome, containing the human antibody gene (Tc bovine). Antibodies generated in non-human primates (NHPs) using a monovalent EBOV vaccine have been shown to cross-react in the current FANG SUDV anti-GP ELISA. There is therefore the potential for background cross-reactivity in individuals who may have been previously exposed to EBOV or SUDV in future outbreaks caused by the other filovirus. Different anti-GP IgG ELISA titres were found with different vaccines and this needs further evaluation, as does possible interference between various antigens in multivalent vaccines.

One of the major lessons learned from the Ebola work was that it is essential to acquire large quantities of critical reagents as early as possible to drive clinical assay development. Once samples are collected, it is important to start stability programs for critical reagents. Clinical and non-clinical samples can either be tested using a single site or multiple sites, and there are *pros* and *cons* to each approach. Single site sample testing is simpler, less resource intense, avoids the need to technology transfer and validation of the assay at multiple sites and offers greater consistency. However, use of a single site increases the risk of bottlenecks in sample testing. Use of multiple sites mitigates bottleneck risks, but requires technology transfer of the assay, necessitates the assay be validated at multiple sites and introduces the risk of inter-laboratory variability. In both scenarios there is a need to advance the design of consent forms (clinical trials) as they should permit use of samples for assays.

MERS-CoV Serology Study

After the first outbreak (or first identification) of MERS-CoV in 2012 a collaborative study was conducted by NIBSC on behalf of WHO. This was based upon the experience with SARS and the need for effective standards. SARS assays had issues with cross-reactivity with normal circulating coronaviruses (which make up more than 15% of “common colds”).

Overall it took five years to gather enough material to run the study. This consisted of five individual samples and three pooled samples, plus Tc Bovine samples. These were collected into groups of high, medium, low and negative responses. In the collaborative study (10 participating laboratories, 24 data sets; qualitative/quantitative) negative and low responses were very similar (low responses were still all confirmed clinical cases) in terms of measured outcome. Samples were also tested against commonly circulating coronaviruses.

Many types of assays were evaluated using these samples. Most negative samples were accurately measured and both high and medium samples were identified as positive. However, the low pool was not detected as positive which, given its similarity to the negative pool, is not surprising. Some individual assays failed to identify certain positive cases, but all labs used algorithms with multiple tests and did identify these cases as positive when considering the full investigation.

NIBSC is considering the use of "Trans-chromosomal" (Tc) Bovine material (see more below) as potential standard material in the absence of sufficient human samples. Therefore, there is some sense that when funding therapeutics it may be useful to leverage samples for standards from the groups developing Tc bovine products. However, as therapeutics and passive immunization are outside of the scope of CEPI, this will likely be through other partners, other groups working in this area or dedicated collaboration specifically to produce standards. Another area of interest relating to this is the use of pooled NHP samples, which could also be useful if available and which should exist at many labs globally; because of a high number of different NHP studies performed in the past.

Clinical Studies for Convalescent Sera

Of the WHO priority pathogens, very few have suitable samples available for the production of international reference standards. A team at the University of Oxford in collaboration with ISARIC (International Severe Acute Respiratory and Emerging Infection Consortium) and NIBSC have proposed a process to address this issue. The proposal involves developing a framework, with processes and template documents that can facilitate the identification of patients, informed consent processes, ethical oversight, benefit sharing, and operational issues such as sample handling. The aim of this collaborative endeavour is to acquire samples from relevant sites in a timely and effective manner. Suitable sources will be identified through a systematic method in order to produce a consistent and reproducible process that can be applied to multiple pathogens. The clinical study protocols will be made available to WG-S&A by ISARIC.

The model seeks to build on the work of existing collaborative projects such as PREPARE and ALERRT. The PREPARE model has 600 hospitals across 35 European countries. ALERRT is a recently funded African clinical research consortium that will include observational and interventional research in multiple sub-Saharan African countries for emerging epidemic threats, including diagnostics.

The ISARIC proposal seeks to establish connections with existing networks in endemic countries and the UK. It will work on ethical consent, which needs to be enhanced to gain individual informed consent and ethical approval for each country and for samples specifically being used as samples not just 'in further research'. Meanwhile benefit-sharing documentation can be built upon existing documents such as the Nagoya protocol allowing equitable access for labs in disease endemic countries. Safe handling will be drawn up individually for each disease, matched to national requirements for UK and donor country. All these processes are planned to start with MERS-CoV, Nipah and Lassa.

Further needs and requirements will be identified by collaborating partners, who will also determine the specific needs for collecting the samples. For the Lassa model this could consist of instituting specific protocols in countries, in advance of the Lassa season, identifying cases, obtaining consent and then obtaining samples once the participants are PCR negative. These samples will have had arrangements made for their safe transport back to the UK as part of the protocol approval process. Other groups are doing serology, sero-prevalence, natural history etc. However, no one else is currently collecting samples such as these for producing standards or reference reagents (nor such large sample size collections exists).

Panel Discussion - How can CEPI best Develop Standards and Assays Fast?

Questions to address;
How will assays and standards support vaccine development?
Who are the key partners in the development of assays and standards?
How should we engage regulators in the assays/standards discussions?

CEPI as a funder is well positioned to guide development of biological standards, harmonized and validated assays and animal models for its priority pathogens. Given the lack of development of standards in these areas there is a clear role for a CEPI Working Group to support the development of standards. The standards will need to be developed rapidly to ensure they are used in the projects already started or very soon to start. Given the state of this area of vaccine development, it is important to recognise the significant need to communicate with regulators and other groups working in this sector. Amongst these other actors, it will be essential to align with academics, manufacturers and other funders to map activities, and minimize overlap and redundancy, and to clearly lay-out a 5-year plan to have a consistent direction.

In particular, it will be important to connect with regulators to ensure alignments. One area of interest would be the European Medicines Agency regulatory pathways. There would still be the question of how one can align (like with a single FDA pathway) when there are so many different sites/countries with different regulatory pathways in Europe?

The Working Group needs to develop a “Master Plan” activities needed and to map the environment relevant for the coming efforts.

Content of “Master Plan”:

- Paths
- Challenges
- Targets
- What can be accelerated
- Overlap with other groups
- Cross-cutting *versus* Specific

Need to Map

a) Normal Pathway
b) Our Proposals

c) Emergency Pathway

Advice needs to be clearly laid out to applicants under CEPI's CfPs; that they need appropriate development of their assays, which don't need to be qualified or validated if these are only used for Phase I clinical trials, though these assays will need more development (e.g. qualification, validation) if the respective vaccine formulations are planned for use through to licensure. Groups will need clarity on passage number for strains, clear documentation as to their origin/provenance (for example whether the sample must come from a fatal case), dose and route (which should follow the natural history of the disease) and other factors. A common issue many groups face is that they may look at duration of protection too late.

The target should be to develop assays, which are:

- Well Characterised
- Qualified (if appropriate*)
- Validated (if appropriate*)
- Handled by experts

* *Appropriate meaning Phase appropriate. Qualified and validated assays are not generally needed to measure immunogenicity in Phase I clinical trials, since these do not typically have primary endpoints.*

Until we have full access to standardized assays and animal models that actually are used in the different development projects, it may be too early for parallel scientific advice, which needs to include countries where proposed products will be used. As a starting point, the CEPI priorities for the next year should focus on animal studies and on use of common standards, assays, and critical reagents where possible, and recommending interim standards, where formal WHO International Biological Standards do not exist. Such interim standards can more readily be established. It is important, that rather than dictate standards, CEPI needs to engage with groups to harmonize requirements and produce common agreements. CEPI will also need to address the question of who will hold and distribute the interim standards.

Animal Models for Lassa, Nipah and MERS-CoV

An Animal Model is more than just a specific animal. It rather a specific combination of animal species, challenge strain and route of administration; that produce a disease process, which resembles important aspects of the human disease. A "challenge" is the harmonization of processes, where many factors of development must be similar to make the challenge agent the same (such as the production method for the viral challenge material). For product developers, an ideal model is one that works best for them; therefore, the model needs to be well characterized before this stage. We also need to know the human disease well before we can establish a good animal model.

Lassa virus (LASV) is highly diverse genetically and biologically. This is especially true for Nigeria, the country with the largest "at risk" population, where at least three genetically different clades of LASV are circulating (Clades I-III). The (ongoing) 2016 LASV outbreak in this country has had a historically unprecedented fatality rate, >50%. Currently, there is very limited information on pathogenicity of Nigerian LASV strains in experimental animals. Almost all animal studies were performed with LASV, Josiah-strain, which is a prototype of Clade IV. Lassa Josiah strain has a

very distinct and stable genotype. This Clade is the phylogenetic home for a large collection of genetically related viruses causing Lassa fever (LF) in Sierra Leone, Liberia, and Nigeria. While LASV is widely diverse genetically and biologically, there is no evidence of direct correlation between genetic clades and clinical manifestations. LASV diversity is a great challenge for vaccine R&D.

Naturally, LASV exists as a persistent form of infection in *Mastomys natalensis*, ("Multi-mammate rat") a rodent species prevalent in sub-Saharan Africa. Rodent-to-human transmission occurs through either inhalation of aerosolized rodent excreta or ingestion of contaminated food. Human-to-human transmission has been documented in nosocomial settings; including non-endemic countries where LASV was imported by infected individuals. In the natural setting, in approximately 80% of individuals LASV causes mild or asymptomatic infection. In about 20% of infected people, especially high risk groups (pregnant women, children under 5 years old), the infection results in severe multi-system LF disease. Pathological findings in Lassa fever patients are very poor even in fatal cases. Lassa virus does not induce cytopathic effects *in vitro* and *in vivo* (in contrast to EBOV). Major histological findings can be found in liver (initially Lassa fever was recognized clinically as Lassa hepatitis) and in other tissues of the reticulo-endothelial system.

The animal models currently used are typically based upon the Josiah strain (which is the primary strain found in most laboratories). However, these laboratories use different techniques to generate "challenge" stocks, which complicates the interpretation of the animal study results. LASV is a rodent-borne virus and NHPs are the only relevant animal model, mimicking human Lassa fever disease. Closely related Rhesus and Cynomolgous monkeys infected with LASV (Josiah strain or related strains from Clade IV) are the most used animal models. Marmosets are also very sensitive to LASV/Josiah and to Nigerian strain 803213 from Clade II (currently the only available NHP model for Nigerian strains). In susceptible animals (e.g. strain 13 guinea pigs) LASV/Jos causes fatal disease by any route of inoculation (including aerosol exposure) at very low dose. LD50 for strain 13 guinea pigs is <1 PFU. Standardization (validation) of LASV/Josiah challenge model and development of animal models for Nigerian LASV strains are the most challenging tasks for LASV vaccine R&D.

Nipah is commonly transmitted by consumption of contaminated food (such as palm sap) and occasionally from contact with body fluids from cases. However, it is unclear whether aerosol transmission plays a role in Nipah infections in the natural settings found in Malaysia, India and Bangladesh. The absence of infections in health care workers (HCW), wearing no or little respiratory protection would suggest it is not common, however RNA detection in throat swabs means it cannot be excluded. The disease is traditionally associated with fruit bats, although many animals can transmit Nipah. Like Lassa it can be observed in clinical presentations as either a severe or a mild disease and has further variation between two main historic outbreak locations (Bangladesh and Malaysia). Mice models are ineffective for Nipah, but African Green Monkeys do mimic human disease, however this has not been established with inhalation as a challenge route.

MERS-CoV transmits from infected people via aerosol transmission (primarily coughing). It is found as a reservoir, in camels. The disease has a spectrum of severity in cases. Mice, Syrian hamsters and ferrets are ineffective models; although NHPs show some resemblance of human disease.

There are no characterized stocks or strains for any of the three priority pathogens. The currently existing assays for immune response, plaque reduction assay, and RT-qPCR are at the research level and the correlates of protection are largely unknown. Animal models are lacking, though there is potential for fairly soon establishment of models for Nipah and Lassa, but less so for MERS-CoV. In all three cases there are also difficulty in giving the challenge dose through the natural route for human infection. An aerosol challenge is difficult, complex and requires a BSL-4 containment with aerosol exposure capabilities for these diseases.

Lassa: Available Assays and Reference Materials

Based on the current ICTV Arenavirus Study Group demarcation criteria, LASV is defined as a species in the genus *Mammarenavirus*. However, classification of LASV strains based on the Pairwise Sequence Comparison (PASC) split currently available strains into six species in line with phylogenetic analysis placing LASV strains at least into four major lineages (Clades). LASV strains circulated in different geographic areas of Nigeria are placed in Clades I-III. The strains isolated from Sierra Leone, Liberia and Guinea are placed in the largest Clade IV, with LASV/Josiah as a prototype. In addition, a fifth Clade has been proposed for recently isolated strains from Mali and Ivory Coast. Historically, most diagnostic tools were developed based on LASV/Josiah. It was documented that up to 32% of anti-LASV antibody positive samples would not have been detected in different West African countries, if only LASV/Jos has been used as antigen. In addition, LASV specific antibodies that are detected in individuals shortly after they recover from Lassa fever, become undetectable after several months, whereas these survivors retained a robust strain-specific and cross-reactive T-cell responses against LASV nucleocapsid protein (NP) and conserved glycoprotein (GP2) epitopes. All these factors contributed to underestimation of LASV prevalence in West Africa. The true incidence of LASV infection is unknown and improved diagnostics are urgently needed. While development of pan-LASV diagnostic kits is very encouraging, the development of reliable and sensitive assays on a regional basis (for LASV prototypes for Clade I-IV) seems to be more realistic approach. Detection of LASV antigens and/or viral RNA copies against IgM-IgG measurement provides more accurate and timely diagnosis. Development of assays to detect LASV-specific T-cell responses (e.g., ELISPOT, ICS) is another priority to accurately assess LASV prevalence in West Africa.

The genetic and biological diversity is a challenge for LASV vaccine R&D. In the revised WHO TPP for LASV vaccine (June 2017), coverage against all major lineages I to IV is proposed. Epidemiological observations in West Africa suggest that in immunocompetent individuals, a single attenuated natural LASV infection results in lengthy protection against fatal Lassa fever disease. Re-infection with heterologous strains provides boosting protective immunity. Indeed, in survived LASV-exposed individuals, strong cross-reactive T-cells responses against NP and GP2 epitopes were detected. These observations provide solid justification for replication-competent vaccine platforms. Currently, based on peer-reviewed publications, cross-protection efficacy against different Clades (IV and II) was documented only for re-assortant ML29; expressing LASV GPC and NP proteins. Recombinant pseudo-virus, VSV/LASV-GPC can protect against

homologous LASV/Josiah and other strains from the same Clade. It seems that inclusion of LASV NP protein in vaccine formulations contributes to effective viral control at early stage of the infection and cross-protection.

Limited information on the pathogenesis of Nigerian strains in experimental animals and the absence of challenge models for Nigerian strains in NHPs (with the exception of LASV from Clade II in marmosets) is another serious obstacle for development of pan-LASV vaccine. In order to make progress, Lassa-specific assays need validation and challenge models need to be developed.

As the 1st step for reference interim LASV strains a prototype for each Clade has to be selected, characterized and establish as an interim reference, for development Clade-specific diagnostics.

The following LASV strains have been proposed as interim standards (based on the Special Pathogens Branch collection, CDC, Bowen et al., 2000):
Clade I: LASV/LP/NIG/69/H (GenBank: AJ969407.1)
Clade II: LASV/803213/NIG/74/H (GenBank: AF181854.1)
Clade III: LASV/GA391/NIG/74/H (GenBank: X52400.1)
Clade IV: LASV/JOSIAH/SL/76/H (GenBank: JO4324.1)

As an option, recent human isolates from Nigeria and other endemic areas can be added to the list.
qRT/PCR assays and SOPs have to be developed as diagnostic standards/protocols for prototypes from each LASV Clade.

The 2nd step for interim references is that challenge strains need to be established and characterized in animal studies. Rhesus or Cynomolgus monkeys must be tested in challenge experiments with Nigerian strains of LASV from Clade I-III with the hope that these animals will be susceptible and mimic human LF. Currently, the only challenge model in marmosets exists for Nigerian strain from Clade II (LASV/803213).

Current pseudo-type viruses; expressing LASV GPC in heterologous genetic backbone (HIV, SIV, VSV) are poor LASV surrogates and can generate false-positive results in neutralization assays . Development of LASV BSL2 surrogates expressing LASV GPC and NP on a genetically-close backbone; LCMV, MOPV or (ideally) the ML29 backbone, has to be a priority and must be considered.

Mapping the current actors in the field and products under development, as well as needs and challenges is an essential starting point for the development of standards for Lassa. The WHO TPP provides some guidance but needs to be considered in the perspective of Lassa fever as a disease caused by genetically distinct viruses which can induce minimal cross-reactive immune responses. A clear starting point for activities will be to map who has what strains, in what state and how well characterized. Then utilising this data, it will be possible to identify strains that could be called standards for each group of Clades. From these, antigens can be identified and named as standards and non-pathogenic surrogate strains in genetically close backbones (LCMV, MOPV, ML29) can be developed to handle these viruses at BSL-2 containment.
Packaged RNA can be used for the PCR validation, but the challenge strains will need to be carefully considered for transport and access. Therefore, when identifying the strains, it will be important to consider national needs, local capacity and other factors to determine whether a strain is suitable for use as a standard.

The strategy and plans for specific manufacturers also need to be considered as this can allow for guidance on validation of assays and assay selection where lacking. Generic advice can be developed for earlier stage proposals.

A substantial number of cases of Lassa fever occur each year, which could serve as sources of antibodies, however many of these cases will be complicated by multiple exposures (also likely with different strains). The serum and PBMC from these cases could be obtained, through proper means, with ethical approval, benefit sharing and other processes in place in advance of a Lassa season. Proper engagement of the groups working on Lassa in Nigeria and Sierra Leone could allow a more expedited and effective route to sample collection. Before doing this, it would be beneficial for the Task Force to build an ideal profile for the samples needed and to provide questions to the groups in the field. In the absence of these samples, recently developed human monoclonal antibodies could be useful as interim standard for LASV from Clade IV.

Nipah: Available Assays and Reference Materials

Nipah virus (NiV) belongs to the *Paramyxoviridae* family and shares the same genus as its close relative Hendra virus with which it serologically cross-reacts.

While the ecological reservoir for both viruses are *Pteropid* fruit bats, the viruses are capable of infecting a wide range of mammalian hosts. The NiV genome is large compared to most Paramyxoviruses and two distinct geographical (geno)types exist; Malaysian (M) and Bangladesh (B). Nipah virus is a BSL-4 pathogen and its mechanisms of pathogenesis remain to be defined. Reverse genetics systems have been developed to study molecular mechanisms of both virus replication and pathogenesis. It is unclear whether aerosol transmission plays a role in Nipah infections in the natural settings found in Malaysia, India and Bangladesh. The near absence of infections in HCW wearing no or little respiratory protection would suggest it is not common. More detailed studies in Bangladesh show transmission primarily from the consumption of virus contaminated palm sap, and in a minority of cases, from close contact with the body fluids of cases. However, aerosol transmission cannot be excluded based on findings such as the detection of virus RNA in throat swabs.

In the Malaysian outbreak of Nipah ran from 1998 to 1999, 283 acute encephalitis cases were reported, with 109 deaths. There was no evidence of human-human transmission in this outbreak and the identified risk factor was contact with pigs. The virus was primarily found in the CNS and pulmonary areas. Subsequent outbreaks were observed in India and Bangladesh. Numerous small outbreaks with a higher case fatality rate (CFR) have occurred from 2001 to the present day. Case fatality rates reached as high as 100% (2011, 28 cases all fatalities) with lows of 25% (4 cases with 1 fatality in 2009) and an overall CFR of 77% over the 10 years from 2001 to 2011 (253 cases and 194 fatalities). Limited human to human transmission has been reported.

There is limited genetic variation among Nipah viruses reaching up to 10% nucleotide diversity in the most variable gene encoding the P protein. To date, complete genome sequences have been released, in GenBank, for 13 virus isolates. A number of RT-PCR assays have been developed for NiV, but few have actually been tested against patient samples. Antibody detection assays, IgM, IgG ELISAs and the microsphere protein array, also have been developed

by utilizing either inactivated NiV cell lysates or recombinant expressed virus proteins as antigens. In addition, neutralization assays have been established based on authentic NiV or genetically engineered pseudo-virions. No rapid diagnostic tests (RDTs) currently exist. IgG ELISA, serum neutralization and qTaqMan RT-PCR will be useful assays to evaluate vaccines under development. For the studies it is important to note that while there is no confirmation of the correlates of protection, existing data suggest that protection correlates with the presence of neutralising antibody. However, the magnitude of antibody response in relation to protection needs further investigation. It may also be important to follow up on the role of cell-mediated immunity as this has been implicated in a few animal studies.

Given the existence of two very different geographically separated Nipah transmissions there may be a need for differing animal models and different viral isolates for challenge. However, for IgG, qTaqMan RT-PCR and virus neutralization the same assay works for both NiV-M and NiV-B.

Authentic Nipah convalescent sera will be ideal for standards. In the absence of sufficient quantities of human sera, it would also be feasible to vaccinate the African Green monkeys, challenge them and use their sera (along with comparative studies). Candidate standards from various studies can also be useful here, such as products produced in CEPI studies. Based on experience with Ebola antibody detection assays, human sera from countries experiencing disease (e.g. Bangladesh) will provide the best assessment of assay specificity and sensitivity. All sera (natural and experimental), could be stored in central repositories for other groups to make use of as deemed necessary.

The use of BSL-2 pseudo-virus for neutralization assays should be investigated, but would need to be rigorously evaluated relative to authentic BSL-4 virus based neutralization assays. Initial priority should be to develop robust IgG ELISA assays.

A detailed study of Nipah natural history must be done to allow the animal models to mimic reality for the route of virus challenge in vaccine studies. Lessons can also draw upon the experiences developing animal vaccines for Hendra. This may help to guide decisions on animal models too, but it will be important to determine who might develop such models and who might fund them. Other areas to follow up include engagement of regulatory authorities to identify whether neutralizing antibody tests will be deemed necessary for approvals. The Nipah virus also needs characterization, both as an ongoing prospect and as a retroactive examination of the viral changes over the past few years, (though significant change is not expected).

MERS-CoV: Available Assays and Reference Materials

The first step to advancing standards for MERS-CoV was deemed to be the mapping of the existing groups working in the area, both in terms of major overarching actors (such as WHO, CEPI, US government agencies, and others) as well as mapping who have and are developing assays, including commercial developers. Having mapped the assay developers, it will be important to prioritise the PCR and neutralizing antibody tests and work from there.

The enabling work of building access to material is the cornerstone of developing MERS-CoV standards effectively. As part of this exercise, it is critical to keep trying to get samples from

Saudi Arabia, as convalescent serum is a gold standard for standard development. Secondary to this, if convalescent serum cannot be accessed, it would be serum from Tc Bovines. It will be important to establish CEPI's role in this, as this technology may need funding but is a large potential resource. This option needs to be evaluated to see if Tc bovine serum is effective as a surrogate in the case of MERS-CoV.

The MERS TPP provides sufficient guidance to go forwards, though the question is raised as to how closely CEPI will follow up on the work connected with development of a camel vaccine option.

Recommendations from 1st Workshop for Task Forces

The Nipah group proposed four task forces:

1. Regulatory (needs an Australian representative)
2. Animal models (needs a USAMRIID representative)
3. Challenge materials
4. Assays

The MERS-CoV group proposed five task forces going forward:

1. Animal Model (cross Lab standardization, speciality in BSL-3/4, challenge virus identification)
2. Lassa
3. MERS-CoV
4. Nipah
5. Sourcing materials

The Lassa group made no specific recommendation with regards to Task Force composition, but noted the need for disease specific landscaping, potential in combination with existing meetings.

Task Forces should consist of people present at the 1st Workshop of CEPI's WG-S&A plus identified topic-experts. Perhaps also regulatory competence and endemic country representatives should be included?

In the process of reiterating the need for an evaluation of actors and products in development, it is important to note this as a cross-cutting need, but also a disease-specific need. Each pathogen will require a workshop to landscape everything that is currently in the concept through to everything in the development stage. It may be sensible to start as soon as possible and to start with the least developed of the priorities. There may also be sense in connecting this process with the WHO's "Roadmap" process(es).

Conclusions and Next Steps

The meeting summarized the current landscape for assays, standards and animal models for the three priority pathogens and Ebola, highlighting gaps and areas where additional research is needed in order to develop vaccines. The meeting developed clear guidance on the way forward

and the next steps for the Biological Standards and Assays Working Group. The Group will continue developing the proposals made so far and initiate specific Task Forces. A clear series of pathways have been proposed to progress different work streams and new areas of work have been identified for future consideration. The Working Group will start work through finalizing its Terms of Reference, development of initial mapping exercises of the existing landscape and through convening follow-up meetings to develop the next stages of work subsequent to the formation of its Task Forces and the mapping exercises.

The Workshop reach a consensus that five Task Forces might be needed. One for each CEPI prioritized disease (Lassa, MERS-CoV and Nipah); one for "Sourcing Materials" and one for Animal Models. Starting up five Task Forces will be quite demanding to handle and co-ordinate at the same time for the CEPI-Secretariat. Thus, it is thought best that the start of the five Task Forces should be staggered; starting with the most prioritized (i.e. Lassa) and wait some with the others. However, it is time for the two co-Chairs to draft mandates, suggest composition/members and elute to deliverables and timelines. A rational and feasible approach might be to start with a Lassa Task Force, followed a bit later by MERS-CoV and Nipah. On the longer run and in a wider perspective; the "cross-cutting" Task Forces for Sourcing Materials and Animal Models will certainly prove valuable to CEPI and the whole field. When these two Task Forces are launched they will profit from the experienced gained from the initial activities in the disease specific Task Forces.

The working group will continue to build on the insight and consensus that has been established during the workshop and in the prior engagement of the members of the working group. The group will expand to bring in expertise relevant to its identified needs, to the new areas of work, to the actors with whom this work overlaps and actors relevant to the changing and evolving environment. However, the group will aim to maintain its small and dynamic format, making use of specific experts as needed, but otherwise limiting growth to essential additions. The various work areas under the Working Group will be developed closely with partners, especially in the WHO R&D Blueprint and for standards with the WHO Expert Committee on Biological Standards (ECBS)

Task Forces will focus in core areas, including cross-cutting groups. These may include regulatory aspects (with careful integration into the broader CEPI Working Group on regulatory aspects), challenge materials, source material access and supply, animal models and assays. These work areas may form into temporary Task Forces, or may be work-areas within a single or a few small multi sectoral groups. The aim of each will be to have a dedicated core working group plan and implement strategies to manage the gaps identified in each area. In order to do this, each group will first map the area. Mapping needs, actors, current work and potential hurdles. After this knowledge-gathering stage a clear Working Plan for the needs will be developed with a path to each objective laid out. The Task Forces will put these into action as work is aligned with appropriate actors and groups for long term and global impact, as well as being brought into the normal CEPI operations for protracted and integrated use of the standards as a normal part of CEPI needs activities.

In addition to cross-cutting groups, one each will be developed for Lassa, MERS-CoV and Nipah. These Task Forces will look at implementing the proposed way forward, adapting this to the changing environment, and will identify both disease-specific and cross-cutting needs in order

to establish standards, norms, and models for their target disease. The disease-specific Task Forces will tackle both the provision of specific guidance to vaccine candidates with already established assays and plans (guiding and validating) as well as a broader and larger focus on the development of generic guidance for unknown or future vaccine candidates. This will include later stage developments on assays, though provisionally the disease-specific working groups will target validation tools for the assays selected by groups. Animal models will be guided in a more specific manner: focusing on a specific animal model that is optimal for evaluating protection against the particular disease and not just getting good read-outs for a specific candidate vaccine.

For each of the three diseases a common pathway has been proposed. First the strains will be considered and key groupings identified (be this by species or by disease pathology). The institutions holding the strains for the pathogen will be identified and amongst them standard strains will be identified for use. These will be dependent on a multitude of factors, including the vaccine and challenge strains by themselves (relative to those in circulation), the method of propagation of strains, and the group who hold the strains, the national requirements regarding these strains, access and ability to share. This will be more complicated when being considered for challenge strains. For PCR these can be shared as packaged RNA, and for other work a surrogate strain (pseudo-virus) which is BSL-2 compatible can be developed. Diagnostics, assays and standards that are currently out there will be considered, and gaps will be identified. With validation strains identified, the next step will be to target antigens (with strains identified this is relatively simple and will be more of an academic exercise on defining the standards for these antigens). The sourcing of antibodies and PBMC will be more complex. Sourcing from convalescent patients (where possible) must be considered ideal or a “gold standard” for serological and T-cell based assays (for LASV), with the ideal model being the use of a protocol to collect PCR negative sera and PBMC from convalescent patients in sufficient bulk to cover a variety of needs. This material must be gathered ethically, with appropriate approvals and with benefit sharing positions in place. Much of this work is being covered by a proposal from ISARIC, along with engagement of regulators and other authorities to allow the samples to be transported across national boundaries. Where new samples are not readily available, pools and existing samples must be considered and new efforts to access them must be pushed forward, secondary pathways must look at alternate models such as Trans-chromosomal bovine sera or non-human primate sera post inoculation and challenge, and these will need to be reviewed in comparative studies if they are used. Specific animal models must be evaluated and validated; as matching the natural history of typical human infection (though this may include multiple models to match multiple pathologies) including infection route, disease progression and appropriate dosing.

While a full International Standard is in development, an interim standard should be developed and recommended for use by product developers and researchers to allow their work to be bridged/undergo comparison and used in models with the International Standards once they become available. Before standards are developed, it would be beneficial to identify who will hold the standards, and who will organize the collaborative studies. Meanwhile newer product developers and newer products after this development should be directed both to these standards but also to previously validated and established assays. Target assays are tertiary to interim models and the development of standards (which can validate and allow comparison of assays used by earlier developers). Once common practice is established, these assays with

standards will allow for greater comparability of future proposed products. Groups must be guided to early engagement with regulatory organizations and to the use of regulatory checklists (especially for those looking to gain approval under the "Animal Rule"). Clear lines of data reporting in standardised ways must be strongly encouraged. Animal models must be defined early and must be based upon offering the best model of human disease, independent of how they respond to the product under evaluation, though these must be selected as relevant to the intervention (including not using otherwise good models, if they are non-responsive to a particular type of intervention where a human would be responsive)

Future areas of work to be investigated by the working group are proposals for the remaining WHO priority pathogens in terms of sample materials assays, standards and animal models. These will be developed in much the same way as for the initial three pathogens targeted already. The same areas of work will also need to be addressed for "Pathogen X", the unknown or unexpected outbreak pathogen for which work has not been established. This will also need to integrate with the work to access material for rapid characterization as part of the product development response and for access to samples from a potentially unprepared region outside of a pre-established protocol (including potentially working with partners to develop access and use agreements in the normal consent forms used for samples taken in these circumstances).

London/Oslo, 12th March, 2018.

"Version 2.0" created by Theo Grace, Elwyn Griffiths and Johan Holst
(Draft version was circulated to all participants in December2017 and January 2018)

LIST OF SPEAKERS and PRESENTATIONS

Richard Hatchett – *Opening Address and Status of CEPI Projects*

Johan Holst – *Introduction of the Working Group and Aims of the Workshop*

Elwyn Griffiths – *Basics of Biological Standards and Standardization*

Melanie Saville – *Expectations of the WG on Standards and Assays from CEPI's Vaccine Development Program and from a Clinical Perspective*

Robin Levis - *Regulatory Requirements to Pre-clinical Data*

Carol Sabourin - *FDA Animal Rule applied on MERS-CoV Vaccine Development*

Larry Wolfraim – *Lessons from Ebola*

Ruth Harvey – *MERS-CoV Serology Study*

Tommy Rampling – *Clinical Studies for Convalescent Sera*

Margaret Louise Pitt *Animal Models for Lassa, Nipah and MERS-CoV*

Igor S. Lukashevich – *Lassa: Available Assays and Reference Materials*

Christina Spiropoulou – *Nipah: Available Assays and Reference Materials*

Johan Holst – *Product Profiles for Biological Standards within the Scope of CEPI's Working Group*

Trygve Danielsen and Emer Cooke – *Summary of the meeting Achievements and Elements for Master Plan for the Working Group Activities*

Appendix

List of Participants

Sina Bavari, USAMRIID, USA.
Emer Cooke, WHO, Switzerland.
Ruth Harvey, NIBSC, UK.
Robin Levis, CBER, FDA, USA.
Francois-Xavier Lery, WHO, Switzerland
Igor S. Lukashevich, Univ of Louisville, USA.
Micha Nüebling, WHO, Switzerland.
Mark Page, NIBSC, UK.
Margaret Louise Pitt, USAMRIID, USA.
Carol Lee Sabourin, Battelle, USA.
Tommy Rampling, ISARIC & Univ of Oxford, UK.
Barbara Schnierle, PEI, Germany.
James Southern, Icon, South-Africa.
Christina Spiropoulou, CDC, USA.
Lawrence A. Wolfraim, NIAID, USA.

Patricia Aprea, (INAME), Argentina (TC)
Eun-Chung Park, NIAID, USA (TC)

CEPI Secretariat

Trygve Danielsen
Theo Grace
Elwyn Griffiths
Richard Hatchett
Johan Holst
Karianne Johansen
Melanie Saville
Solomon Abebe Yimer

Nicole Lurie (TC)

Agenda: Biological Standards and Assays Workshop

Date: ***12th December 2017***

Location: ***Wellcome Trust, London, UK*** ***In general 15 min talks + 3-5' Q&A***

08:30	*Coffee and Tea available*
CHAIR:	**Emer Cooke,** WHO and co-Chair of WG-S&A
09:00 (10 min)	**Opening Address and Status for CEPI Development Projects –** Richard Hatchett, CEO of CEPI
09:10 (10 min)	**Introduction of the Working Group and Aims of the Workshop –** Johan Holst, CEPI
09:20 (15 min)	**Basics on Biological Standards and Standardization –** Elwyn Griffiths, CEPI
09:40 (15 min)	**Expectations to the WG on Standards and Assays; from CEPI's Vaccine Development Program; from a Clinical Perspective –** Melanie Saville, CEPI
10:00 (15 min)	**Regulatory Considerations for Biological Standards and Assay Development –** Robin Levis, CBER, USA
10:20 (15 min)	**FDA Animal Rule; Applied on MERS-CoV Vaccine Development –** Carol L. Sabourin, Battelle, USA
10:40 (20 min)	*Break with Coffee and Tea available*
CHAIR:	**Elwyn Griffiths,** CEPI
11:00 (15 min)	**Lessons from Ebola –** Larry Wolfraim, NIAID, USA
11:20 (15 min)	**MERS-CoV Serology Study –** Ruth Harvey, NIBSC, UK
11:40 (15 min)	**Clinical Studies for Convalescent Sera –** Tommy Rampling, ISARIC, University of Oxford, UK
12:00 (20 min)	***Panel Discussion; How can CEPI best Develop Standards and Assays fast?*** *Questions to address; How will assays and standards support vaccine development? Who are the key partners in the development of assays and standards? How should we engage regulators in the assays/standards discussions? Melanie Saville, CEPI, Sina Bavari, USAMRIID and Larry Wolfraim, NIAID*
12:20 (50 min)	*Lunch*
CHAIR:	**Johan Holst,** CEPI
13:10 (15 min)	**Animal Models for Lassa, Nipah and MERS-CoV –** Margaret Louise Pitt, USAMRIID, USA
13:30 (15 min)	**Lassa: Available Assays and Reference Materials** – Igor S. Lukashevich, University of Louisville, USA
13:50 (15 min)	**Nipah: Available Assays and Reference Materials** – Christina F. Spiropoulou, CDC, USA
14.10 (15 min)	**Product Profiles for Biological Standards within the Scope of CEPI's WG –** Johan Holst, CEPI
14.30 (10 min)	*Break*
14:50 (2 hrs)	**Divide into three groups – Lassa, Nipah and MERS-CoV; Review Product Profiles, Draft Recommendations and Suggest Deliverables (**1:15 in Groups + 45 min Plenum)

16:50 10 min	*Break*
17:00 (30 min)	**Summary of Workshop Achievements and Elements for a Master Plan for the WG's Activities –** Trygve Danielsen, CEPI and Emer Cooke, WHO
17:30	**End**
17:45 **(30 min)**	**Guided Tour at Wellcome Collection & 18:15-19:00 Individual Study of the Collection**
19:00	**Joint Dinner (optional): At the Wellcome Trust; *in house* Chef**