

Developing Biological Reference Materials to Prepare for Epidemics

[Announcer] This program is presented by the Centers for Disease Control and Prevention.

[Sarah Gregory] Hi, I'm Sarah Gregory, and today I'm talking with Dr. Tommy Rampling, in the U.K. Dr. Rampling is an infectious disease clinician and academic clinical fellow at the Hospital for Tropical Diseases and University College in London. We'll be discussing his article about the need for biological reference materials to prepare for high-threat pathogens. Welcome, Dr. Rampling.

[Tommy Rampling] Hi, Sarah. Thanks so much for having me.

[Sarah Gregory] So, I'm going to start off reading your abstract. I don't normally do this, but in this case it contains a lot of terms that we want to discuss today. So, this is an important topic. With globalization, the threat of deadly diseases is continuing to grow.

Recent years have seen unprecedented investment in research and development for countermeasures for high-threat pathogens, including specific and ambitious objectives for development of diagnostics, therapeutics, and vaccines. The inadequate availability of biological reference materials for these pathogens poses a genuine obstacle in pursuit of these objectives, and the lack of a comprehensive and equitable framework for developing reference materials is a weakness. We outline the need for internationally standardized biological materials for high-threat pathogens as a core element of global health security. We also outline the key components of a framework for addressing this deficiency.

Dr. Rampling, what are biological reference materials and how are they used?

[Tommy Rampling] So, biological reference materials are standardized complex macromolecules that have a defined unit of activity or potency. When they are available, it allows for consistency in comparison across assays and between laboratories globally. So, they have important applications in the standardization of serology assays used in epidemiology and vaccine efficacy trials, and in nucleic acid amplification tests. These are assays where biological activity can't be characterized by physical or chemical means alone. And the use of reference materials also insures the consistency of production and quality of biological medicinal products, such as monoclonal antibodies, and it's essential for the establishment of appropriate clinical dosing of these products.

So, international reference preparations are considered to be the gold standard of reference materials, and they provide a common set of reagents against which regional, national, and international laboratories and manufacturers can then calibrate their own working standards. So, reference materials only become WHO-endorsed international reference preparations after extensive collaborative studies involving multiple laboratories internationally. And this process allows the materials to be ascribed defined units of biological activity, most commonly international units. So, there are hardly any international reference standards available for high-consequence infectious diseases with epidemic potential, such as Lassa fever, MERS coronavirus, or Nipah virus. Availability of reference materials for these high-threat infections

would be a major asset in the development and evaluation of new diagnostics, vaccines, and biotherapeutics for these diseases.

[Sarah Gregory] What's the history of biological standardization and what does it involve?

[Tommy Rampling] The concept of biological standardization first came about in the late 19th century, and it's linked to the discovery of diphtheria antitoxin. Diphtheria antitoxin is made by harvesting diphtheria toxin from the bacteria and injecting it into horses and retrieving the horse serum, which should hopefully be rich in antitoxin antibodies. After it was discovered, France successfully recreated the process, but attempts in England produced, essentially, an ineffective product. And this failure was thought to be due to weak serum and it led Paul Ehrlich, in Germany, to propose the use of a standardized antitoxin preparation which would be measured in units and with which you could calibrate future batches and therefore ensure potency.

So, after that, in the 1920s, the concept was applied to other biological products, such as insulin. But there was recognition for the need of some kind of international oversight. So, in the 1920s, the League of Nations initiated the Provision of International Reference Preparations, under the Commission on Biological Standardization. And then biological standardization was subsequently incorporated into the constitution of the WHO when it was formed in 1946.

Since then, the provision of WHO reference materials has played a vital role in facilitating the translation of laboratory science into worldwide clinical practice. And this has mainly been delivered through the WHO Expert Committee on Biological Standardization, for which there is an annual meeting to establish detail recommendations and guidelines for the manufacturing, licensing, and control of various biological standards.

[Sarah Gregory] Would you give us some examples of high-threat pathogens.

[Tommy Rampling] So, high-threat pathogens would be those that have an ability to cause an acute infectious disease, an ability to spread within communities and within healthcare settings. They usually have a high case-fatality rate, or morbidity. They're often difficult to detect and identify rapidly and there's often a lack of specific treatments.

So, in 2015, in the wake of the West African Ebola epidemic, the WHO launched the R&D Blueprint for Action to Prevent Epidemics. And this is a global strategy and preparedness plan for dealing with high-threat pathogens with the potential to create a public health emergency. Central to the R&D Blueprint is a list of priority diseases, which is reviewed through a tailored prioritization process on an annual basis, with the most recent being in February 2018. So, the current list of R&D Blueprint priority pathogens includes Crimean Congo hemorrhagic fever; filoviruses, such as Ebola and Marburg; Lassa fever virus; Severe respiratory coronaviruses, such as MERS and SARS; Nipah virus; Rift Valley Fever virus; Zika virus; and then they have a space at the end for Disease X. Disease X isn't actually a disease, but it's included to represent the knowledge that a serious international epidemic could be caused by a pathogen as yet unknown to cause human disease. And so, the R&D Blueprint explicitly seeks to enable cross-cutting R&D preparedness; it's also relevant for an unknown Disease X, as far as possible.

[Sarah Gregory] What's the status of vaccines and other therapeutics for use with these high-threat pathogens?

[Tommy Rampling] So, the status of currently licensed vaccines or therapeutics for these diseases, or indeed drugs and vaccines for which there is a substantial evidence base demonstrating efficacy, is very poor at the moment. However, one of the positive outcomes to emerge from the tragedy of the West African Ebola virus outbreak, was a renewed focus in investment and initiative to expedite R&D, with an aim to ensure that we are better prepared for future disease outbreaks. So, this includes initiatives like the WHO Blueprint and it also includes allied initiatives, such as CEPI, the Coalition for Epidemic Preparedness Innovations, whose aim is to accelerate the development of vaccines for high-threat pathogens. So, this has led to a significant amount of investment into vaccine development programs for these diseases. But also into other related fields, such as predictive modeling to preempt epidemics, rapid response initiatives, and clinical trial design to attempt to answer the complex question of how best to assess the efficacy of drugs and vaccines in intensely complex settings, such as in the midst of an outbreak of a high-threat disease.

So, there is now an Ebola virus vaccine that is supported by some limited efficacy data, and this is being used on a compassionate use basis in the current Ebola outbreak in the Democratic Republic of Congo, with over 70,000 contacts of Ebola now having been vaccinated. There are many questions still to be answered about this vaccine, however, and the accelerated clinical development in 2014–2015 was only in response to the occurrence of a large-scale outbreak. In terms of drugs, Ribavirin is used for Lassa fever and CCHS. However, it's not exactly a new drug and the supporting efficacy data are still limited. There are many questions that still remain around its use. Otherwise, there's no other vaccines or therapeutics in routine use for those diseases on the R&D Blueprint.

[Sarah Gregory] Talk to us a little bit about the challenges in developing these standards.

[Tommy Rampling] So, antigen- and nucleic acid-based reference standards are often a bit more straightforward, as these can be synthesized through recombinant techniques, providing you have the necessary genomic sequence data. For completely novel emerging pathogens, however, a collection of samples of live pathogens may be required, which adds a significant layer of complexity. The production of antibody-based reference preparations is a bit more difficult. So, the ideal starting material for these is serum or plasma from convalescent patients. This is not a huge problem for many common infectious diseases. So, for example, if you take parvovirus B19, a very large number of people who contribute to the National Blood Transfusion Service in the U.K. will be serum positive. Therefore, a tiny fraction of the serum held by the service can be donated to the producers of international reference standards, such as NIBSC, which is the U.K. National Institute for Biological Standards and Control, and a standard can then be produced.

It gets much more complicated when the disease in question is far less prevalent, when the occurrence of that disease is sporadic, and when patients are more difficult to identify and sample. So, novel methods for generating fully human immunoglobulin for use as reference materials have been explored when human plasma is not readily available. This includes techniques, such as the inoculation of transchromosomal cattle and the subsequent retrieval of antibodies. But this technology is still relatively new and convalescent human serum is considered superior because it will most closely represent that clinical sample and it has a polyclonal range of antibody specificities, which can then be further optimized by pooling samples. So, in addition to the challenge of actually obtaining samples, the production and

validation of these standards takes a considerable amount of time. So, once the actual reference preparation has been made, they then need to be validated in extensive collaborative studies. And this whole process, from the receipt of the starting material to the WHO endorsement as an international standard, usually takes two to three years.

[Sarah Gregory] Is it difficult to get patient samples?

[Tommy Rampling] So, for the diseases on the R&D Blueprint, yes, it is difficult; it's complicated by several factors. Firstly, you have the issue of identifying the patients who would be suitable to donate blood for the production of standards. Many of the pathogens on the R&D...the WHO priority pathogens list cause outbreaks, so they're difficult to anticipate in both time and place, and the outbreaks are often limited in both numbers of cases and duration. So, these factors complicate the prospect of acquisition of the appropriate clinical samples. And, in addition, these pathogens most commonly cause outbreaks in resource-constrained environments, where poor healthcare infrastructure, limited diagnostic capability, and suboptimal disease reporting systems will make the retrospective identification of suitable patients difficult. It's also important to mention that simple blood draws can significantly complicate field operations in the midst of an epidemic and logistical issues around the processing and storage of acute, potentially infectious samples can also present many obstacles.

So, an international antibody standard for Asian lineage Zika virus was made available by NIBSC very recently. And the only other disease on the R&D Blueprint, for which there are currently international antibody reference standards available for, is Ebola. And for the development of these standards, plasma was obtained from recovered Ebola virus disease patients, either from Sierra Leone, who were enrolled in a convalescent plasma trial, or as donations from countries not directly involved in the 2014 outbreak, such as the U.K. and the U.S. But it would be inappropriate to use this as a model for obtaining samples because it was dependent on the occurrence of a very large-scale and devastating outbreak with a concerted international response in order to be successful.

So, focused efforts to obtain material should be made with methods to identify and sample patients from prior outbreaks being explored, in addition to preparations for promptly identifying and sampling patients prospectively diagnosed in future outbreaks. And this would require a systematic and coordinated approach that brings together local investigators with international partners in a transparent and equitable framework. This could be done through the sharing of clinical research tools, such as open-access clinical study protocols and coordination of the collaborative framework to outline processes for obtaining samples. And preemptive identification study sites and preparation of study documents, such as template protocols and clinical agreements, would hopefully optimize the likelihood of success.

[Sarah Gregory] Are there ethical considerations involved in this goal—issues like consent and such?

[Tommy Rampling] So, there are issues around ethics and consent. The process of acquiring new samples for the development of reference materials should be registered as a unique study for each prioritized epidemic disease or written in as an objective into an appropriate existing or planned study. This would then require ethical approval being sought through the appropriate channels, allowing for scrutiny by the relevant ethical and regulatory bodies, both in the

countries coordinating the activities and in the disease-endemic countries where samples are to be collected. These studies would involve minimal risk to participants as they wouldn't involve the administration of experimental therapies and, in most cases, only a single blood draw would be required.

But informed consent would be an indispensable requirement for donation. The donor should receive information concerning all aspects of this study, with emphasis being placed on the fact that participation is entirely voluntary. The intended use of the blood components for the production, storage, and distribution of international reference materials should be explained. And this could all be done in detailed participant information sheets that would be provided to the donor in advance of providing consent. And then the usual consent processes would be necessary, of participants signing and dating informed consent forms before any study-specific procedures are performed. But a collaborative framework could include template participant information sheets and consent forms that could be rapidly and readily adapted for disease- and country-specific studies, when appropriate.

[Sarah Gregory] How would these samples be shared and, ultimately, who owns them?

[Tommy Rampling] So, the sharing of biological materials is necessary for rapid research progress. But recent experiences during epidemics have highlighted that we need to make concerted efforts to establish acceptable processes for obtaining and sharing reference materials. At present, only a small number of institutions manufacture and provide reference materials to the WHO for use as international reference preparations. So, export of samples to these institutions is currently an essential step in their production.

There are examples of existing initiatives that aim to facilitate the sharing of benefits that arise from the use of and access to genetic materials. One example is the pandemic influenza preparedness framework, which was launched by the WHO in 2011. This is a framework that seeks to address the concerns of low- and middle-income countries, that their sharing of influenza virus specimens with the WHO was not then matched with assurances that any benefits that were derived from these shared samples would be equitably distributed.

These concerns about inequitable sharing of benefits were then exacerbated further during the 2009 H1N1 pandemic on account of the unequal access to vaccines. And there have been more recent epidemics of other infectious diseases that have continued to raise issues with regards to sample sharing, such as the local export restrictions in Brazil that prevented the sharing of well-characterized samples during the Zika virus epidemic.

Further generalized guidance on the fair and equitable sharing of benefits arising out the use of genetic biological resources can be found in the Nagoya Protocol. This is a supplementary agreement to the Convention on Biological Diversity and contains detailed guidance on access obligations, benefit and sharing obligations, and compliance obligations. Although there is no similar guidance for plasma-derived products, such as antibody reference materials, many facets of the Nagoya Protocol would remain pertinent to plasma-derived products. And these could be adapted to inform procedures.

The WHO oversees the distribution of international reference preparations, either free of charge to national control laboratories or with small shipping and handling charges to other

organizations. Procedures ensuring compliance with the principles of equitable benefit sharing and solutions to complex issues, such as intellectual property, product ownership, and access rights, should be devised through negotiations between relevant parties, and preemptive interaction between researchers and donor country authorities. These processes could subsequently be specified in legally binding clinical study agreements.

[Sarah Gregory] And what about logistical considerations? How would these highly contagious samples be housed and transported?

[Tommy Rampling] So, there are definitely significant logistical considerations here that need some forethought. So, most of the diseases on the R&D Blueprint priority list are caused by Category A pathogens. And any handling of potentially infectious materials would require Biosafety Level 4 laboratories, which although increasing in number globally, are still relatively rare. Recently, concerns over bioterrorism has resulted in strict rules and requirements, such as the U.S. federal Select Agent Regulations, that can result in increased cost, limited research, and reduced collaboration between institutions.

As I mentioned, however, there has been an encouraging and significant growth in the number of Biosafety Level 4 facilities globally. And initiatives now exist that seek to harmonize practices and facilitate collaboration between these laboratories. The safe collection, processing, exporting, and importing of samples from patients who have recovered from high-consequence infectious diseases would require careful planning, with requirements based on the natural history of the specific disease, the study site, the patient characteristics, such as how long they've been in convalescence since the acute illness.

It would be important to liaise closely with national authorities in both the donor and recipient countries with regards to specific exportation and importation regulations and requirements. In addition, most of these diseases occur primarily in low- and middle-income countries and often in remote or rural settings where access to accurate medical records and diagnostic tools would be limited. So, clear specifications regarding documentation and sample provenance would also be required.

[Sarah Gregory] I know we've covered some of this, but what's the ultimate end goal here and what's the real benefit to public health and to individuals?

[Tommy Rampling] So, international reference preparations for these high-threat infections with epidemic potential are vital for the rapid development of vaccines, therapeutics, and diagnostics. So, they're therefore an important asset to strengthen global health security. In our article, we've outlined a number of notable barriers to the development of these reference materials, not least the collection of suitable source material, and we've highlighted a series of key issues that we suggest need to be addressed systematically in a framework that is acceptable to all parties. We've proposed that work should begin to develop and agree such a framework and to generate reference preparations for these diseases so that drugs, diagnostics, and vaccines are available for future outbreaks.

[Sarah Gregory] Tell us about your job and what interests you about infectious diseases.

[Tommy Rampling] So, I'm an infectious diseases and virology academic clinical fellow, currently working at the Hospital for Tropical Diseases in London, U.K. So, we have an

outpatient and inpatient service for tropical medicine and for general infectious diseases. My research background is in clinical trials, specifically of Ebola and malaria vaccines. And my other interests are in severe viral infections and imported fever. Infectious diseases is just so interesting to me because it's constantly changing, it's multisystem, it's influenced by so many complex factors, and it's international. And it's for these reasons that I love it.

[Sarah Gregory] Thank you so much for taking the time to talk with me today, Dr. Rampling.

[Tommy Rampling] Thank you so much for having me.

[Sarah Gregory] You, our listeners, can read the February 2019 article, International Biological Reference Preparations for Epidemic Infectious Diseases, online at cdc.gov/eid.

I'm Sarah Gregory for *Emerging Infectious Diseases*.

[Announcer] For the most accurate health information, visit cdc.gov or call 1-800-CDC-INFO.