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Perspective

Recommendations for Publication of Viral Genetic Data and Sample Access for Novel Viruses and Strains

Nicole C. Arrigo, Thomas Briese, Charles H. Calisher, Michael A. Drebot, Brian Hjelle, James W. LeDuc, Ann M. Powers, Patricia M. Repik, John T. Roehrig, Connie S. Schmaljohn, Robert B. Tesh, and Scott C. Weaver*

Center for Infection and Immunity, Mailman School of Public Health, Columbia University, New York, New York; Arthropod-Borne and Infectious Diseases Laboratory, Department of Microbiology, Immunology and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado; Viral Zoonoses, Zoonotic Diseases and Special Pathogens, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba, Canada; Infectious Diseases and Inflammation Program, Departments of Pathology, Biology, and Molecular Genetics and Microbiology, University of New Mexico School of Medicine, Albuquerque, New Mexico; Institute for Human Infections and Immunity, University of Texas Medical Branch, Galveston, Texas; Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado; Virology Branch, Division of Microbiology and Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland; Department of Molecular Virology, Virology Division, United States Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, Maryland

Reference collections of viruses and virus strains representing their temporal, geographic, and phenotypic ranges are critical to basic research and public health. Such collections have proved essential for helping to determine the sources of new outbreaks as well as studying viral pathogenesis, taxonomy, emergence, and evolution. The development and validation of new diagnostics, therapeutics, and vaccines with appropriate breadth of coverage also rely on comprehensive collections of virus strains for validation studies. Despite their critical importance, many reference collections now struggle to maintain contemporary virus isolates from across geographic and host ranges. As stated by Robert Shope, who for many years, maintained the World Reference Center on Arboviruses at Yale University and later at the University of Texas Medical Branch, "Virus collection has virtually ceased. We need to find a politically acceptable way to return to the collecting business, perhaps in the name of basic science or preservation of biological diversity."[1] This trend is the result of reduced virus isolation efforts and the regulatory burdens that many countries now require to share or transfer certain viruses to appropriate repositories. For example, in the United States, the U.S. Department of Agriculture (USDA), Centers for Disease Control and Prevention (CDC), and sometimes Convention on International Trade in Endangered Species (CITES) of Wild Fauna and Flora permits are required for the importation and/or transfer of many viruses or even host and vector samples; in addition, Commerce Department permits are required to export many viruses, sometimes even to endemic countries of their origin. For regulated select agents, an extra layer of permitting and security is involved. Delays caused by these permitting and compliance processes can have devastating consequences if they impact the exchange of materials needed to aid in the research and public health responses to disease outbreaks.

There are multiple reasons for the decline in virus isolations by different sectors of the biomedical research and public health communities. The development of molecular genetic technologies during the past 3 decades has dramatically changed the ecologic and epidemiologic study of known viruses as well as the detection and identification of known or previously unrecognized viruses. Before the 1980s, the detection of viruses in diagnostic or surveillance samples generally relied on the inoculation of animals or cell cultures. Virus isolation was followed by serologic tests to characterize the viral antigens using antisera raised to the new isolate and other previously characterized virus strains. Virus isolates were commonly placed into collections at many research and diagnostic laboratories and readily shared, with few regulatory hurdles. Virus assays were also developed to detect directly some viral antigens in diagnostic or surveillance samples, but their sensitivities vary and are generally less than direct culture.

With the discovery of reverse transcriptases (RTs)[2,3] followed by the development of the polymerase chain reaction (PCR),[4] specific and sensitive tests to detect the presence of viral RNA (RT-PCR) or DNA (PCR) were developed for many virus groups. As genomic sequence databases grew, it also became possible to design (RT-)PCR assays to detect broader groups of viruses at the genus or family level. Using these methods, it is now possible to detect the presence of many known and unknown viruses of well-characterized taxa through (RT-)PCR assays, often with comparable sensitivity to virus isolation. However, these assays remain a challenge for some of the largest and most diverse virus groups, such as the bunyaviruses, rhabdoviruses, and reoviruses, for which sequences of many members are still lacking. These assays are also limited by the availability of amplifiable RNA in a sample, such as serum or other body fluids that may contain virus for only a short window of time during acute infection. Therefore, although the inoculation of animals that develop disease or cell cultures that develop cytopathic effects remain the best methods to detect viruses in some poorly characterized groups, some of these systems do not consistently support viral replication, result in overt disease, or generate cytopathic effects.

More advanced, deep, metagenomic sequencing of samples containing viruses is sometimes capable of detecting presently unrecognized or unculturable viruses. However, limitations in the types of samples available, technical skill requirements,

*Address correspondence to Scott C. Weaver, 301 University Blvd, Institute for Human Infections and Immunity, University of Texas Medical Branch, Galveston, TX 77555-0609. E-mail: sweaver@utmb.edu

throughput, and cost all restrict the use of this technology in routine applications. Alternatively, the use of (RT-)PCR offers several advantages over virus isolation for the detection of viruses in diagnostic or surveillance samples compared with virus isolation. (1) Reagents and equipment are widely available, and assays can be performed at low cost. (2) There is no need for cell culture systems, laboratory animals, or antisera collections for antigenic characterization of virus isolates, which are not available universally or optimized for all virus groups. (3) Nucleic acid extraction for genetic analysis often inactivates infectious virus, eliminating the need for high levels of biosafety and security. (4) Direct sequencing of PCR amplicons allows for the rapid (although sometimes superficial) genetic characterization of viruses present in a sample to precisely characterize and genetically type the viral sequences present.

For these reasons, many research and diagnostic laboratories now rely exclusively on genetic detection and analyses of viruses, and genomic sequence data are often the only information available to other investigators. This shift to genetic detection and characterization without virus isolation has important consequences and implications for virology research and the integrity of the scientific process. The virus classification system implemented through the International Committee on the Taxonomy of Viruses explicitly defines taxa based on polythetic criteria,[5] and therefore, genetic sequences alone may not be adequate for taxonomic designation. More importantly, the lack of a virus isolate or access to an original sample can preclude the opportunity for scientists to further characterize a virus, including critical studies of virulence, pathogenicity, transmissibility, and/or vector infectivity. Also lost is the ability to repeat and validate the genetic assays used in published studies for virus detection and identification, which undermines a cornerstone of the scientific process of peer evaluation. Furthermore, the reporting of only partial genomic sequences can reduce the accuracy of phylogenetic studies and preclude the comprehensive evaluation of reassortment or recombination, which can be critical to understanding virus evolution, host range, pathogenesis, and replication. Finally, the thorough validation of new diagnostic assays and the testing of vaccines and therapeutics require contemporary virus isolates representing wide-ranging human or animal exposure to ensure broad coverage.

Regulatory constraints can also impede virus isolation efforts, especially for those viruses designated as select agents, because of the maze of compliance hurdles associated with culturing, maintaining, or transferring highly pathogenic agents or samples to registered facilities with appropriate biocontainment and security. Detection of a restricted agent may result in the destruction or inactivation of the original sample, which may contain an important historical or public health threat virus, thus precluding additional investigation and thorough phenotypic characterization. Also, although the sharing of reagents is usually a requirement of research funded by the US National Institutes of Health (http://grants.nih.gov/grants/policy/nihgps_2010/nihgps_ch8.htm#_Toc271264947) and a condition for publication in most scientific journals, some researchers are reluctant or unable to share materials because of fear of competition or the burden of regulatory requirements.

Technological advances in molecular genetics have provided numerous scientific benefits. However, overreliance on these techniques coupled with ever-increasing regulations impedes progress in virology research and challenges our scientific integrity. Although it would be unrealistic to require research entities to perform virus isolation on all samples in which viral nucleic acid is detected, there are some relatively simple and inexpensive measures that could be implemented to facilitate the isolation and retention of important virus strains. Transfer of an aliquot of the original diagnostic or surveillance sample and/or the virus isolate to qualified repositories such as reference center collections supported by the National Institutes of Health and other funding agencies should be facilitated. If original samples are consumed during the initial assays, the retention of amplified genetic material for resequencing or more detailed characterization by other investigators should also be strongly encouraged. The cooperation of journal editors could help with these efforts by encouraging the deposit of genetic sequences into public databases and adherence to these guidelines as a condition for final publication, much like the current requirements of many journals to explicitly note access to reagents or deposit into public databases of genetic sequences. Regulatory agencies should also be urged to simplify and standardize the transfer process in their issuing of importation or exportation permits, including transfer approvals for restricted agents. The development of easily transferrable credentials for handling regulated agents would also be helpful in streamlining research efforts. Ultimately, these or other measures designed to increase access to original samples or derived genetic materials are needed to uphold scientific standards, protect the future of experimental virology, and improve preparedness for future viral emergence by providing comprehensive virus collections for outbreak investigations, basic research, and product development.

As members of the Subcommittee on Inter-Relationships Among Catalogued Arboviruses (SIRACA) of the American Committee on Arthropod-Borne Viruses, we urge the development of logistical support systems, the revisitation of regulatory requirements, and the consideration by journal editors of publication standards that will reverse the decreasing availability of field samples and virus isolates that is undermining progress in research on many viral diseases.

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Authors' addresses: Nicole C. Arrigo and Thomas Briese, Center for Infection and Immunity, Mailman School of Public Health, Columbia University, New York, NY, E-mails: ncarrigo@gmail.com and thomas.briese@columbia.edu. Charles H. Calisher, Arthropod-Borne and Infectious Diseases Laboratory, Department of Microbiology, Immunology and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, E-mail: calisher@cybersafe.net. Michael A. Drebot, Viral Zoonoses, Zoonotic Diseases and Special Pathogens, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba, Canada, E-mail: Mike_Drebot@hc-sc.gc.ca. Brian Hjelle, Infectious Diseases and Inflammation Program, Departments of Pathology, Biology, and Molecular Genetics and Microbiology, University of New Mexico School of Medicine, Albuquerque, NM, E-mail: bhjelle@salud.unm.edu. James W. LeDuc, Robert B. Tesh, and Scott C. Weaver, Institute for Human Infections and Immunity, University of Texas Medical Branch, Galveston, TX, E-mails: jwleduc@UTMB.EDU, rtesh@utmb.edu, and sweaver@utmb.edu. Ann M. Powers and John T. Roehrig, Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, CO, E-mails: AKP7@cdc.gov and tr1@CDC.GOV. Patricia M. Repik, Virology Branch, Division of Microbiology and

Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, E-mail: prepik@niaid.nih.gov. Connie S. Schmaljohn, Department of Molecular Virology, Virology Division, United States Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, MD, E-mail: connie.schmaljohn@us.army.mil.

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