



# Methods for fighting emerging pathogens

We asked scientists around the globe for their views on the basic research methods, tools and resources needed to fight future emerging pathogenic threats.



**Luiz Carlos Junior Alcantara:** The COVID-19 pandemic has reshaped the world in a variety of ways, leading to millions of cases and deaths globally, economic decline, disruption to

the social fabric and many other hardships. This emphasized that country-level strategies for pandemic preparedness need to be put in place throughout the world. Recently we have established a mobile national network in Brazil, called VigECoV-2, for real-time genomic sequencing, genomic epidemiology and data analysis in public health laboratories. We believe that this methodology can be a powerful toolkit for quickly identifying emerging viral threats, monitoring the evolution of their genomes, understanding the origins of outbreaks and epidemics, predicting future outbreaks and helping to maintain updated diagnostic methods through the generation of data that can inform outbreak control responses.



**Lucas Amenga-Etego:** Nanopore sequencing technology has been critical in tracking SARS-CoV-2 spread and identifying variants. Key challenges

have been to increase the sensitivity for samples containing low concentrations of RNA (with  $C_t$  values  $>25$ ), and to innovate and develop robust assembly tools to understand how these samples, which currently fail to be detected through sequencing, may influence viral evolution and spread. It may also be useful to attempt primer-free sequencing approaches with Nanopore sequencing methodology to make experiments cheaper and faster. Additionally, mitigating the effects of future pandemics will require robust, high-throughput model systems and organisms to use to fully understand host immune response and identify viral entry inhibitors and neutralizing agents.



**Robin Andersson:** Enhancers play a key role in activating and maintaining functional phenotypes for controlling disease. Better understanding of the mechanisms through which

enhancers regulate gene activity may therefore provide crucial insights into disease pathogenesis and, ultimately, prevention or treatment. For this, new assays are needed that can capture regulatory activities in single cells, at large scale and with high sensitivity, allowing the establishment of probabilistic models of transcriptional regulation based on the stochasticity of regulatory events. From there, interpretable and generalizable computational models need to be devised that allow inference of the determinants of regulatory activity of individual enhancers and the functional interplay of multiple enhancers within regulatory domains.



**Moumita Bhaumik:** Much progress has been made in the field of vaccine research during the COVID-19 pandemic. However, the efficacy of these vaccines in immunocom-

promised individuals remains uncertain. Therefore, there is a need to develop delivery systems that will better serve immunocompromised subjects. Use of trained immunity or innate immune memory can be exploited to cause such individuals to mount more robust immune response. Expressing the antigen of interest in a trained-immunity-based vector, such as Bacillus Calmette–Guérin (BCG), will help to stimulate both innate and adaptive immune memory and will enhance the efficiency of vaccines. With the addition of cytokines as adjuvants and with delivery by liposomes or nanoparticles, BCG-based vaccines will open up new avenues of exploration for the generation of vaccines against pandemic pathogens.



**Young Ki Choi:** Following the flu and COVID-19 pandemics, the continuous outbreaks of novel viral infections underscore the threats of the next potential pandemic, the so-called ‘Disease

X’. However, a vast number of viruses cannot be studied well in vitro due to the lack of readily available culture systems for predicting viral infectivity. Organoids, which mainly represent cultured tissue derived from stem cells, could be a valuable tool for studying viruses that are difficult to culture in vitro. The physiological and structural similarities of organoids to specific tissues or organs provide also better resolution of virus–host interaction dynamics that are invisible in two-dimensional systems. Given these advantages, organoid-based culture systems will be invaluable for studying the as-yet-unknown new pandemic candidates that will succeed SARS-CoV-2.



**Hélène Decaluwe:** The past decade has seen an extraordinary burst of knowledge about T-cell-mediated immune responses to pathogens, thanks to the development of powerful flow and mass cytometry approaches combined with single-cell transcriptomic

Credit: CHU Sainte-Justine – Stéphane Dedelis

pipelines and T cell receptor (TCR) sequencing approaches. These technological advances have revealed the diversity and heterogeneity of the T cell pool following infections and have shed light on the cellular and molecular parameters shaping effective and long-lasting immune responses in the host. Developing high-throughput technologies to assess T cell responses to viruses of pandemic potential in large cohorts of subjects efficiently will be essential for the effective assessment of the next pandemic. Further, we will need to develop accessible bioinformatic pipelines to efficiently and reliably identify immunogenic epitopes based on identified TCR sequences.



Credit: Sharron Bennett Photography

**Jemma Geoghegan:**

The rapid appearance and spread of this virus highlights the devastating consequences of viral emergence following a host jump. Although host-jumping is the most important source of emerging viruses, the process

is poorly understood: we have only a limited understanding of the mechanisms that enhance or inhibit virus emergence. A major factor contributing to this shortcoming is the narrow, biased and fragmented nature of existing virus sampling. To spot and stop emerging viruses, we require insight into how the natural ecological characteristics of hosts and viruses facilitate viral diversification and spread. We should now exploit advances in genomics to better understand the drivers of virus emergence, augmenting our ability to prevent and contain emerging infectious diseases.



**Bart L. Haagmans:**

The COVID-19 pandemic has emphasized the need for accurate in vitro systems to rapidly assess the antigenicity, transmissibility and pathogenicity

of newly emerging viruses. Organoids, three-dimensional in vitro structures grown from stem cells, may allow such phenotypic characterization. Biobanks of organoids derived from several tissues or organs and reflecting genetic diversity in humans, together with CRISPR technologies and advanced proteomics, represent methodological advances to help us quickly decipher the biology of newly emerging viruses. Combining cell biology and virology expertise may thus ultimately reveal genotype–phenotype relationships in great detail and lead to effective vaccines and therapies to combat newly emerging viruses.

**Susana López:** One of the bottlenecks during the COVID-19 pandemic has been the combination of long test turnaround times and limited access to sensitive and accurate diagnostic methods. Thus, the development of diagnostic methods with the sensitivity and reliability of the reverse transcription quantitative polymerase chain reaction (RT-qPCR) that provide faster results and that are suitable for use at point



of care, require less sophisticated equipment and are more widely accessible is—and will continue to be—a crucial need in many parts of the world in future pandemics. For

example, developing more robust and cheaper loop-mediated isothermal amplification (LAMP) tests will greatly accelerate diagnostics. Further to this, the development of high-throughput methods to screen for pre-existing antibodies in human sera, to make it possible to perform routine serological surveillance of populations and determine individuals' previous contacts with pathogens, will be very useful in establishing the immune state of a given population, particularly during epidemic or pandemic events.



**Maowia M. Mukhtar:**

The successful use of mRNA vaccines to reduce morbidity and mortality from SARS-CoV-2 has opened a new era for the use of mRNA as an interventional

molecule. The challenge in using mRNA vaccines is the emergence of new pathogens or variants of the same pathogen that are immunologically different, thus requiring booster doses or development of new versions of the mRNA vaccine. The future focus for mRNA vaccine research will be to develop polycistronic mRNAs that translate multiple epitopes to generate antibody response to different pathogens or to variants of the same pathogen.



**Erni Nelwan:**

The COVID-19 pandemic has been a lesson in how newly emerging pathogens can cause disruptions on a global scale. One of the biggest roadblocks in our response to this

pandemic has been the high quality of big data that is needed to design biological studies. In preparation for future pandemics, access to data should be shared in a fair and ethical manner between nations, regardless of economic status. For example, we and others have developed the Randomised Evaluation of COVID-19 Therapy

(RECOVERY) Trial, which focuses on engaging countries to find effective solutions for COVID-19 using an adaptive trial approach. Nations should also revise their regulations regarding the sharing of and access to biological data to facilitate broad access to data.



**Elias A. Rahal:**

The COVID-19 pandemic has demonstrated how valuable the One Health approach (which recognizes that the health of people is closely connected to the health of animals

and our shared environment) is to the identification of emerging pathogenic threats. Changes affecting the biology of viruses circulating in humans, as well as in natural reservoirs such as bats or livestock, may easily result in the next global health challenge. This pandemic has been a lesson on the importance of pathogen monitoring for health authorities worldwide. Proper surveillance of pathogens will be required to curb the next pandemic early on. Sequencing of viral genomes for surveillance purposes will hugely aid this effort because other techniques may be more cumbersome and expensive. However, for sequencing to take its proper place in this equation, it must become cheaper and more available, particularly in communities that lack resources and technical training.



**Kei Sato:**

Transmissibility, pathogenicity, and resistance to immunity and drugs—the SARS-CoV-2 pandemic let us know that these three concerns

are the frontline questions with emerging viruses and need to be investigated and revealed in real time. To achieve this, interdisciplinary collaborative investigations will be indispensable. For instance, in an effort to help combat SARS-CoV-2 pandemic, I launched the Genotype to Phenotype Japan (G2P-Japan) Consortium. The G2P-Japan Consortium consists of not only experimental virologists and clinicians but also experts in bioinformatics, statistics and molecular phylogenetic. We hope that such interdisciplinary approaches (we call this 'systems virology') to examining multiple aspects of emerging viruses will be the standard for future virology and play a

crucial role in helping us get ahead of future pandemic threats.



**Ella H. Sklan:** Major progress in viral surveillance and sequencing methods has made it possible to rapidly detect emerging viral variants, thus enabling countries to respond to these

variants of concern appropriately. Despite these advances in variant detection, methods to infer the functional effects of the various accumulated mutations still lag and depend on decades-old, slow neutralization assays. New algorithms for predicting protein folds and protein–protein interfaces will enable better prediction of the phenotypic and thus immunological consequences of such mutations and help flag variants of concern with vaccine evasion potential. Furthermore, a basic understanding of the functional consequences of such mutations might promote the rapid design of specific vaccines limiting the spread of such variants.

**Yvonne Su Chuan Fang:** Viral phylodynamics has advanced our understanding of the emergence and spread of endemic and novel diseases. Combating infectious diseases is challenging, and early detection and characterization of



emerging pathogens are important but require an unbiased, systematic and highly responsive surveillance system. Despite significant improvement in pandemic response during the

COVID-19 pandemic, further advances in sequencing technologies, data science and artificial intelligence applications are needed for rapid generation, sharing and analysis of genomic data. New tools and expertise for studying the evolutionary dynamics of virus populations at both inter-host and intra-host levels are critically important to fully capture the complexity of rapidly evolving pathogens and to track real-time evolution of novel variants over time and space.

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