Labcorp's perspective: Responding to SARS-CoV-2 and the next pandemic

The magnitude of the COVID-19 pandemic has tested the limits of the response capacities of governments, public health and healthcare infrastructure, and diagnostic laboratories. Over the first year of the pandemic, more than 100 million cases of COVID-19 have been recorded, with more than two million deaths. A critical component of an effective pandemic response is diagnostic testing capacity, and the pandemic continues to demonstrate the need to have this capacity with precision and at scale. Commercial laboratories can be important partners in an effective pandemic response. Labcorp stands out for its ability to deliver scientific expertise, perform more than 275,000 polymerase chain reaction (PCR) tests for SARS-CoV-2 on a daily basis, perform viral genomic sequencing, serologic and neutralizing antibody assays, and provide a full complement of drug and vaccine development capabilities.

Infectious diseases are likely as old as life itself. As humans, we may owe our very existence to infection of a primitive cell by a protobacteria, giving rise to a cell with an energy source—a mitochondrion. Life in ancient Egypt was complicated by malaria, tuberculosis and schistosomiasis, which remain endemic today. The Plague of Justinian, beginning in 541 AD, was a devastating outbreak of bubonic plague, that claimed perhaps a quarter of the eastern Mediterranean population. Further waves of bubonic plague, beginning in 1347, devastated the European population. Although at the time the 'Black Death' was thought to be an act of God, there was a rudimentary concept of contagion. In 1377, the Venetian-controlled port of Ragusa imposed a 30-day isolation period for ships entering port. This practice was adopted by other cities and eventually extended to a 40-day period, called a *quarantino*, from which the word quarantine derives[1]. Smallpox and other imported pathogens devastated indigenous populations in the Americas from the 16th century onward.

Past pandemics had important sociological and geopolitical consequences, and they have also left evolutionary fingerprints. Some poxviruses are able to bind to CC-chemokine receptor 5 (CCR5) and gain entry into human cells. Following a northern European poxvirus outbreak centuries ago, a 32-base pair deletion in the CCR5 gene became established in the population. This protective mutation persists today. It affords partial protection from infection with HIV, which uses CCR5 as a co-receptor for entry into human CD4+ T cells[2].

Influenza pandemics over the last century demonstrate the vulnerability of the human population to viral diseases. In the modern era, increasing population density, major perturbations of complex ecosystems and global travel have proved to be efficient kindling for zoonotic viral infections[3]. Although the human immune system has evolved to be able to recognize virtually any pathogen *de novo*, some viruses with rapid generation times and error-prone nucleic acid polymerases will invariably emerge through their ability to co-opt normal human cell surface receptors for entry.

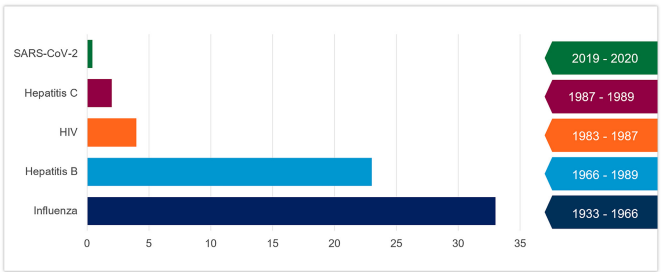

Figure 1. Time from discovery of virus to first approved/authorized treatment

Over the last 4 decades, emerging and re-emerging viruses, including HIV, the Sin Nombre hantavirus, Zika, Ebola, Chikungunya, Nipah, Hendra, and coronaviruses, including SARS-CoV, MERS-CoV, and now SARS-CoV-2, have highlighted the need for speed in responding to new pathogens. Fortunately, advances such as next-generation sequencing, single cell sorting, nanotechnology, and bioinformatics have enabled faster discovery of pathogens and faster development of diagnostics, therapeutics, and vaccines.

Thirty-three years elapsed from the discovery of the influenza virus in 1933 until approval of the antiviral drug amantadine. For hepatitis B, interferon-alpha was approved for treatment in 1989, or 23 years after discovery of the virus. The rapid discovery of HIV just two years after the first published clinical descriptions of AIDS, and the approval of AZT just 4 years later marked a turning point. In the case of SARS-CoV-2, a novel coronavirus was isolated and sequenced only weeks after reports of a new severe acute respiratory syndrome in Wuhan, China (https://www.ncbi.nlm.nih.gov/nuccore/MN908947.1). The antiviral drug remdesivir received emergency use authorization within 5 months of publication of the viral genome (**Figure 1**). Remarkable progress was made throughout 2020 developing diagnostic tests, therapeutics and vaccine candidates, all related to scientific and technological innovations that enable essential components of pandemic responsiveness.

DIAGNOSTICS

The starting point for an effective pandemic response is the tools to identify the pathogen. SARS-CoV-2 is a case in point, as infection may be asymptomatic or may mimic symptoms associated with influenza or other respiratory illnesses. Just 13 days after the SARS-CoV-2 genome was posted online, a

multinational collaborative group published a validated RT-PCR workflow for the sensitive and specific detection of the virus[4]. Seven weeks after publication of the SARS-CoV-2 genome, the U.S. FDA updated guidance relating to the development of *in vitro* diagnostic testing during the pandemic, and the first emergency use authorization (EUA) for a commercial RT-PCR diagnostic test was granted two weeks later to Labcorp. Serial enhancements, including automation and conversion to a multiplex format, allowed for an unprecedented increase in test volume capacity, which now exceeds 275,000 per day. As of December 31, 2020, Labcorp had provided more than 30 million SARS-CoV-2 PCR test results, representing one of the largest numbers from any single entity in the U.S. Labcorp's performance demonstrates that commercial laboratories operating on a large scale with rigorous standards can be a critical component of a robust pandemic response.

Miniaturization, microfluidics and other technological innovations have also enabled rapid turnaround point-of-care (POC) nucleic acid amplification and antigen-based tests. Serologic tests for SARS-CoV-2 were developed quickly following publication of the viral genome. Important work on MERS-CoV provided a framework enabling production of recombinant spike protein stabilized in its native pre-fusion trimeric state[5]. Highly sensitive and specific serologic tests have been developed on a variety of platforms, including enzyme immunoassay and chemiluminescence. Rapid lateral flow-based tests are also available; however, the performance of these tests varies widely.

Although the correlates of protective immunity following natural SARS-CoV-2 infection or vaccination are not yet fully defined, early data indicated that emergence of neutralizing antibodies to the spike protein, and to the receptor binding domain (RBD) in particular, correlate well with convalescence. Pseudovirion-based chemiluminescent fusion assays were developed and implemented by Monogram Biosciences, a Labcorp specialty testing laboratory to study HIV-neutralizing antibodies nearly 20 years ago[6] (**Figure 2**), and this technique has been adapted for other viral targets, including SARS-CoV-2. This assay, which obviates the need for other assays that require live virus, yields valuable information about vaccine-induced immune responses.

Nucleic acid sequencing is another critical tool for tracking and responding to the COVID-19 pandemic. Consistent with the fact that the virus is a rapidly replicating RNA virus with an error-prone RNA-dependent RNA polymerase, many genetic polymorphisms arise over time. The vast majority of these have to date proven useful for tracking the spread of the pandemic, but clinically relevant mutations are exceedingly rare so far[7,8]. A cluster of cases beginning in September 2020 in England have a new viral variant in common, known as B.1.1.7, or VOC-202012/01 [https://virological.org/t/preliminary-genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-the-uk-defined-by-a-novel-set-of-spike-mutations/563]. Epidemiologic data suggest that this virus is rapidly becoming predominant at a regional level, possibly related to an increase in viral fitness and infectivity. Continued surveillance is critical so that biologically important variants (e.g. variants that may be less susceptible to neutralization by vaccine-elicited antibodies) can be detected and appropriate measures taken.

THERAPEUTICS

The discovery of HIV and elucidation of its replicative cycle led to a robust pipeline of antiretroviral drugs. These were first targeted to the viral reverse transcriptase, inhibiting replication. The addition of inhibitors of the viral protease and integrase, as well as inhibitors of cellular fusion and entry have radically altered the landscape of antiviral therapy and the prognosis for HIV-infected individuals. These discoveries have fueled antiviral drug discovery and development more broadly in recent years, including, for example, curative antiviral treatments for hepatitis C virus infection (**Figure 3**).

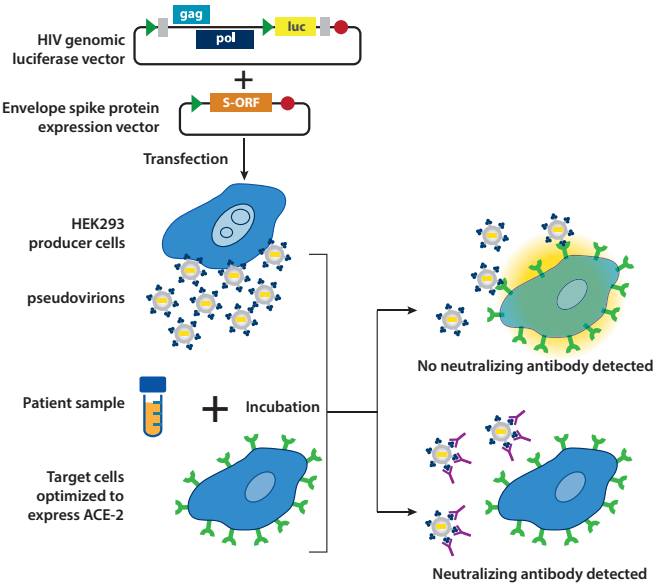

Figure 2. Schematic of assay for the detection of neutralizing antibodies to the SARS-CoV-2 spike protein.

Several broad-spectrum antiviral agents, including remdesivir, were in development when the COVID-19 pandemic emerged. Remdesivir, an adenosine analog pro-drug, binds viral RNA-dependent RNA polymerase, resulting in RNA chain termination and inhibition of viral replication. Clinical trials using remdesivir for the treatment of COVID-19 began in February 2020; it received EUA in May, followed by full approval from FDA in October.

EIDD-2801 (now molnupiravir) is an orally available pro-drug of the nucleoside analogue N4-hydroxycytidine (NHC) that was also discovered to have potent *in vitro* activity against SARS-CoV-2. Although NHC is a nucleoside analogue, it does not act as a chain terminator during RNA transcription. Because the molecule can tautomerize, it may base pair as either cytosine or uracil, thus disrupting the fidelity of transcription resulting in viral 'error catastrophe'[9]. In a remarkable effort, the first-in-human study began in April 2020 and phase III testing will begin early in 2021.

Therapeutic monoclonal antibodies can now be isolated, screened and produced relatively rapidly because of advances in single cell sorting technology. Individual immunoglobulin expressing B cells that bind to the SARS-CoV-2 spike protein or its receptor binding domain can be isolated, heavy and light chain cDNAs can then be cloned and transfected into mammalian cells to produce monoclonal antibodies. A combination of casirivimab (REGN10933) and imdevimab (REGN10987), two monoclonal antibodies that recognize different epitopes of the SARS-CoV-2 receptor binding domain, as well as bamlanivimab (LYCoV555), a monoclonal

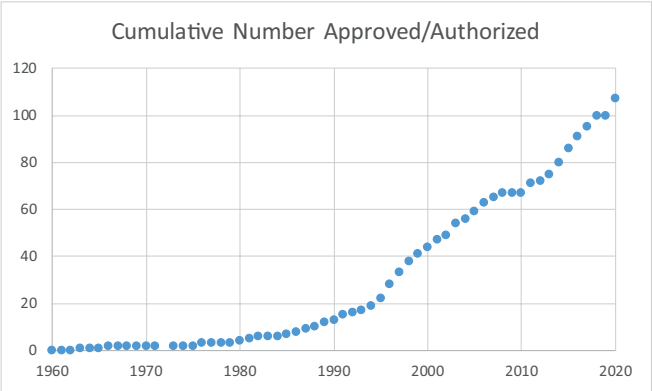

Figure 3. Cumulative number of FDA-approved or authorized antiviral drug treatments, 1960-2020.

antibody that recognizes the viral spike protein, have already received emergency use authorization from the FDA.

VACCINES

Nothing illustrates the remarkable pace of scientific progress more than the development and deployment of multiple vaccines to prevent COVID-19 within a year of the discovery of the virus. As of December 22, 2020, there were 172 vaccine candidates in pre-clinical and 61 in clinical development spanning the entire spectrum of vaccine technologies, including live-attenuated virus, inactivated virus, protein subunits, virus-like particles, viral genes expressed by viral vectors, DNA, and RNA. Many lessons learned from past research were immediately applicable to SARS-CoV-2 vaccine design, because its spike protein is a class I fusion glycoprotein analogous to those of other beta-corona and respiratory viruses and represents the primary target for neutralizing antibodies.

The first two vaccines (BNT162b2 and mRNA-1273) to receive EUA from UK, US and EU regulatory authorities are both RNA vaccines. Scientists have been trying unsuccessfully for decades to make this simple idea work – deliver a messenger RNA that will enter cells and be translated and processed into the relevant antigen. Two of the largest hurdles included delivering a sufficient quantity of mRNA into cells and getting the translated protein to assume the desired, native conformation to optimally induce a protective immune response. Part of the delivery problem was solved by use of lipid nanoparticle packaging. The other breakthrough was the discovery that substituted nucleosides incorporated into synthetic mRNA allow the molecule to enter cells in stealth mode, without triggering an innate immune response[10]. As noted above, more recent work solved the problem of ensuring that the translated spike protein would assume its native configuration once translated. Altering the mRNA sequence to encode two rigid proline residues at key positions within the spike protein allows the translated protein to assume its native pre-fusion trimeric structure[5].

THE FUTURE

As monumental an achievement as it was to develop multiple vaccines within a year, immunization of billions of people is only the beginning of the end of the SARS-CoV-2 pandemic. Follow-up of vaccinated individuals will be essential in order to identify actual and surrogate determinants, as well as the durability of protective immunity. It would be difficult to scale the pseudovirus neutralizing antibody assay to the tens to hundreds of millions level, and therefore surrogate markers of this activity (i.e., perhaps quantitative anti-RBD IgG levels) will be key management tools.

The emergence of the B.1.1.7 viral variant with numerous mutations, including ones affecting the spike protein, demonstrate the importance of constant genomic surveillance of virus isolates. Emergence of new strains that are more transmissible, cause an increase in disease severity, or that are not susceptible to neutralization by an immune response elicited by currently available vaccines should inform public health policy and clinical decision-making. It remains to be determined whether new vaccines will need to be made and delivered periodically, as is the case for influenza.

As much as has been learned about SARS-CoV-2 in the initial year of the pandemic, there remains much to learn about the pathogenesis of the disease that it causes, COVID-19. Viral and host factors that determine the severity of disease have just begun to be understood. The complexity of COVID-19 clinical syndromes (e.g., pneumonia, thrombosis, myocarditis, acute kidney injury, multisystem inflammatory syndrome, cytokine storm and others) is daunting.

How prepared are we for the next pandemic? The periodic antigenic shifts that lead to influenza pandemics are constant reminders of the need for vigilance and preparedness. In hindsight, the SARS-CoV-2 pandemic should not have been so surprising. SARS-CoV and MERS-CoV had already demonstrated that highly pathogenic coronaviruses could emerge as zoonotic infections, and data published in 2015 from studies in bats revealed the presence of coronaviruses with the potential to infect human cells[11]. Urbanization, deforestation, climate change and continents interconnected by migration and travel will continue to provide opportunities for pathogens to be introduced to human populations through animal and insect reservoirs[12]. Science remains the most valuable asset in our toolkit to identify and respond to the next pandemic, and Labcorp will continue to be a steward of science, providing support, innovation, and leadership to help reduce the next pandemic's impact.

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