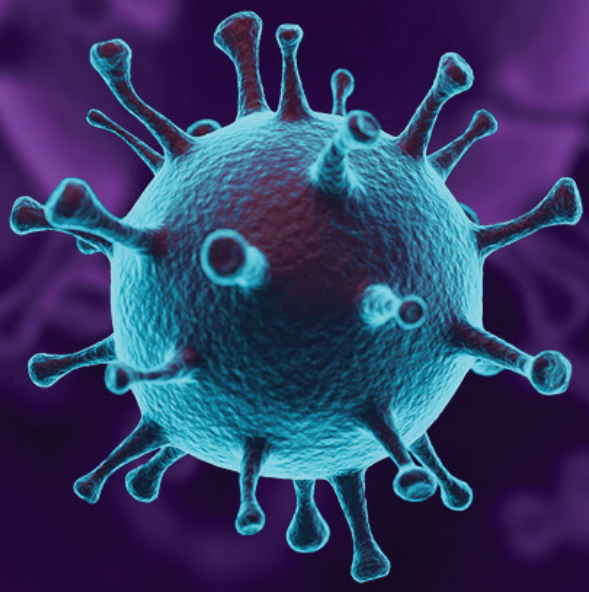
DISCOVERY LIFE SCIENCES™

Biospecimen & Biomarker Services

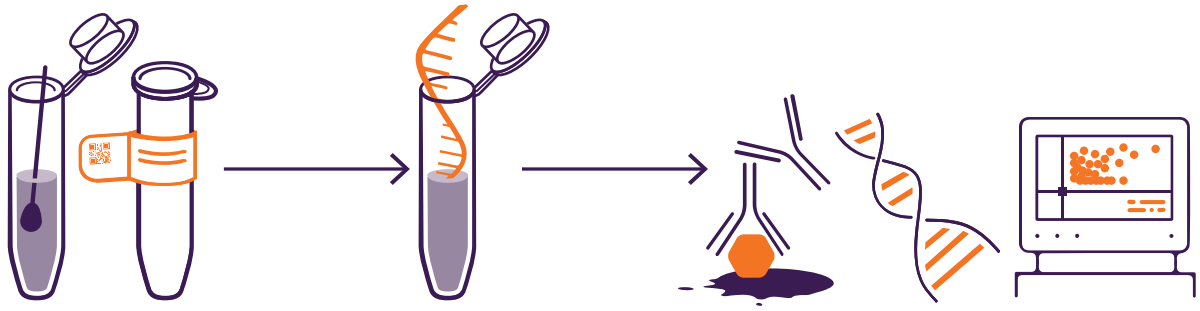
to Advance SARS-CoV-2 Research for the Development of COVID-19 Diagnostics and Targeted Therapies.



SARS-CoV-2, the novel coronavirus that causes COVID-19, has exacted an enormous toll across the world, causing severe disease, death, and disruptions to society.

In an effort to track, mitigate, and control the impact of the virus, unprecedented global scientific efforts have been initiated to develop new diagnostics, targeted therapies, and vaccines. All of these efforts require the collection of vast amounts of data from individuals and populations infected by SARS-CoV-2.

Discovery Life Sciences is on the forefront of the global scientific fight, providing biospecimens and biomarker data to help scientists understand the virus itself, such as how it spreads, infects, and evolves new variants, as well as helping researchers develop and validate new diagnostic tests, vaccines and therapies.



SARS-CoV-2 Biospecimen

Optimized Nucleic Acid Isolation Services

Biomarker Services

- Transcriptional insights into the virus and host response
- Surveillance and tracking of viral variants
- Sensitive viral detection

Biospecimens

PROVIDING NATURAL, CLINICAL SAMPLES TO POWER DIAGNOSTIC & VACCINE STUDIES

Researchers need access to significant volumes of SARS-CoV-2 biospecimens, spanning a variety of sample types, to power large studies and produce the data needed to advance the understanding, diagnosis, vaccination and treatment of SARS-CoV-2. Discovery offers the largest commercial inventory of remnant positive SARS-CoV-2 samples, fully consented or matched positive SARS-CoV-2 biospecimens, in addition to normal controls and and positive biospecimens for other respiratory illnesses. Through our global network of clinical sites, Discovery Partners®, Discovery has hundreds of thousands of samples and can conduct prospective collections of nasopharyngeal, nasal and throat swabs, whole blood, plasma, serum, PBMCs and saliva from patients around the world.

Our samples include key information such as demographic characterization of the patient (e.g., age, gender, ethnicity), medical and treatment history, details related to the testing method, result, and date of acquisition, and clinical outcomes. Discovery's biospecimen collections adhere to all applicable guidance from the CDC and the WHO, with positive and negative results determined by CLIA-certified labs, that allow us to support COVID-19 diagnostic, vaccine, and therapeutic studies. This is particularly important as the FDA diagnostic template recommends using natural clinical specimens in the development and evaluation of diagnostic assays (FDA, 2020). Patient samples contain the natural biological complexity that artificially manufactured samples lack, but which real-world detection methods must be able to manage.

Diagnostic Studies

Differential comparisons with negative control samples, or between SARS-CoV-2 and other respiratory viruses, were conducted using Discovery's specimens collected from healthy subjects and patients with pneumonia, adenovirus, influenza, rhinovirus, and others, and helped in the development of more rapid, sensitive, and accessible diagnostic testing (Byrnes et al., 2021; Steiner et al., 2020; Arumugam et al., 2020).

Biospecimens provided by Discovery were also used to develop improved methods of SARS-CoV-2 RNA detection, where affinity-capture hydrogel particles help concentrate SARS-CoV-2 and substantially improved RT-PCR signals to eliminate false negatives (Barclay et al., 2020).

Importantly, the ability to prospectively or longitudinally collect these samples across nearly 300 clinical sites around the world means that Discovery can partner with investigators in the experimental design and discovery stages to support large and on-going studies.

Vaccine Studies

Biospecimens from Discovery played a pivotal role in BioNTech's "Project Lightspeed" to successfully and quickly develop their SE COVID-19 vaccine (BioNTech, 2020).

Immunohistochemistry (IHC) performed on formalin fixed, paraffin embedded (FFPE) tissues from cases confirmed to contain Mycobacterial infections has supported the utility of the Bacillus Calmette-Guerin (BCG) vaccine for possible protection from SARS-CoV-2 infection. This was based on the high homology between a SARS-CoV-2 envelope protein and a Mycobacterium bovis consensus protein, and evidenced by the cross-hybridization of a SARS-CoV-2 envelope antibody with the M.bovis protein. These data indicated that BCG vaccination can induce immediate and specific immunity against SARS CoV-2 by targeting the viral envelope protein that is essential for infectivity (Nuovo et al., 2020a).

Biomarker Services

GENOMIC, FLOW CYTOMETRY & IHC APPLICATIONS

Beyond sample acquisition and provision, Discovery offers a deeper understanding of the samples themselves by providing high-quality genomic and IHC data. This includes optimized dual DNA/RNA isolation services from SARS-CoV-2 positive and negative biospecimens (e.g., swabs, saliva, stool, blood, tissue, sewage, environmental swabs, etc.). Across these sample types, Discovery has experience with tens of thousands of samples and achieves a 98% sequencing success rate using proprietary isolation services and optimized workflows.

Pathophysiology Applications

Formalin-fixed paraffin-embedded (FFPE) tissue samples can be a rich source of information on SARS-CoV-2, and Discovery Life Sciences has deep expertise and novel methods for extracting the highest quality material from challenging samples. Optimized workflows at HudsonAlpha Discovery allow for dual extraction of RNA and DNA from FFPE tissue that translates into valuable RNA-Seq data (Discovery Life Sciences, 2020). These data can be integrated with orthogonal technologies like immunohistochemistry (IHC) to build a more complete understanding of the biological impact of the virus.

For example, Discovery has optimized IHC staining for the COVID-19 spike and envelope protein and COVID-19 RNA can be detected in situ. This allows for co-expression detection of the COVID-19 proteins with other targets of interest. Cytologic preparations obtained from SARS-CoV-2 patients have shown that the virus targets the glandular cells in the nasopharynx and that productive infection can be found both there and in the lungs (Nuovo et al., 2020b). Furthermore, autopsy tissues from individuals who had severe COVID-19 infections show that COVID-19 can present as a multifaceted viral-triggered vasculopathy syndrome affecting multiple organ systems (Magro, 2021). For example, in situ detection of infectious SARS-CoV-2 and viral capsid proteins along with the cellular target(s) and host response determined that the SARS-CoV2 virus activated the complement pathway and coagulation cascade. This activation can result in increased blood clotting and the expression of cytokines that lead to a cytokine storm (Magro, 2021). It also appeared that degenerating virus releases the capsid protein in the circulation, which can dock with the angiotensin converting enzyme 2 (ACE2) positive expression in the endothelia of the brain, heart, skin, and liver to cause substantial or perhaps fatal disease (Magro, 2021). The information gleaned from embedded tissues samples has provided insight into SARS-CoV-2 infectivity and COVID-19 pathophysiology.

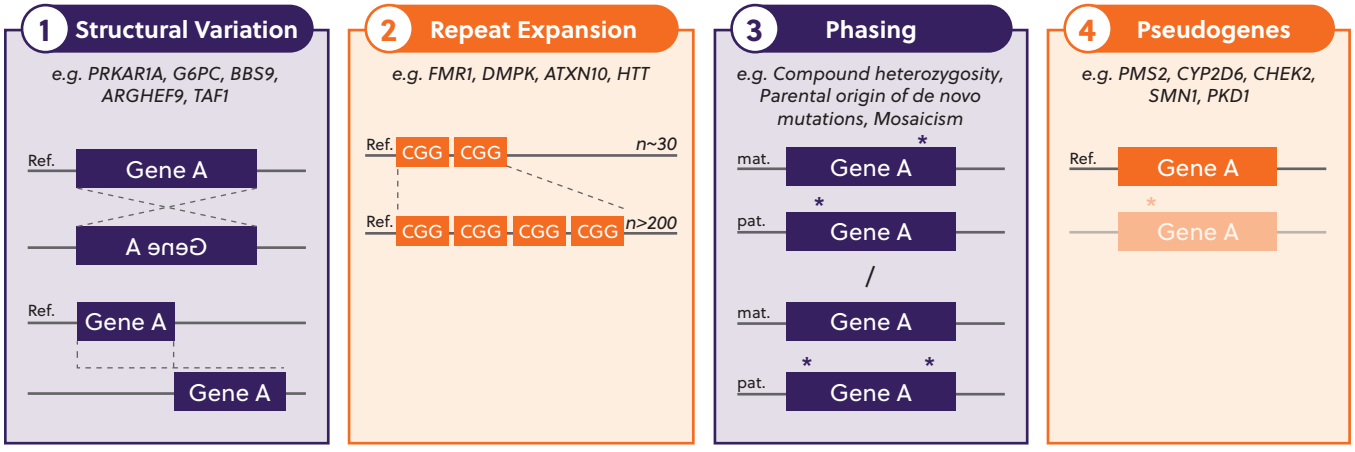
Faster, More Reliable and Sensitive Viral Detection

Using a rapid, validated loop-mediated isothermal amplification protocol (LAMP), SARS-CoV-2 can be identified from patient samples, and proceeded by a large-scale, total-RNA-Sequencing for host, bacterial, and viral profiling. Total RNA-Seq services, including sequencing for both mRNA and miRNA, offer accurate identification of SARS-CoV-2 variants and more descriptive viral surveillance. LAMP, along with RT-PCR, has become a means of efficiently detecting viral genes, offering faster reaction times and increased sensitivity (Yüce et al., 2021). Working with our partners and clients, Discovery has applied these technologies across hundreds of specimens to define new features of SARS-CoV-2 evolution and highlighted the limitation of the qRT-PCR approach to SARS-CoV-2 detection, by identifying false negatives (Butler et al., 2020).

Coronaviruses contain 4 main structural proteins: nucleocapsid (N); spike (S), which is critical for binding to host cell receptors and infection; envelope (E), which interacts with the membrane to form the viral envelope; and membrane (M) that determines the shape of the viral envelope and organizes the CoV assembly (Seah et al., 2020). Mutations in these structural proteins can alter the infectivity and transmissibility of the virus but they may also bias variant detection, since the S gene may drop out during PCR analysis of some strains (Washington et al., 2020; World Health Organization, 2020). Therefore, while short read sequencing allows for high throughput, long read sequencing (LRS) on platforms like PacBio and Oxford Nanopore Technologies at HudsonAlpha Discovery can robustly determine genetic variants and mutations to fully characterize a strain of choice. For example, the PacBio platform reduces errors in sequencing and can sequence unique genomic loci that short reads are unable to accomplish. Efficient and diverse sample collection, combined with rapid testing and thorough profiling of the infectious agents, can support diagnostic development and new therapeutic targets for SARS-CoV-2 and other viral outbreaks.

HudsonAlpha Discovery's PacBio Based LRS Services

- Uses Single Molecule Real Time (SMRT) technology
- Typical insert sizes are 10-150+ kb (vs. 50-250+ bp read lengths using short read sequencing technologies)
- Use of Circular Consensus Sequencing (CCS) where each molecule is read repeatedly to reduce error (also known as HiFi)
- The DNA template does not undergo PCR prior to being sequenced
- Errors are stochastic and can be overcome with deeper sequencing of the same or multiple DNA molecules
- Sequence unique genomic loci often not possible using short reads including examples described below



Understanding the Virus and Host Response

Total RNA-Seq, along with rapid LAMP testing, has helped profile both the virus and host response. Viral sequencing data of the SARS-CoV-2 genome have been used in a phylogenetic analysis to define viral subclades and help epidemiologists understand community spread and virus evolution (Butler et al., 2020). Total RNA-Seq can also provide insight into the unique host response by analyzing the differential expression of host genes. These analyses have uncovered unique commonalities to SARS-CoV-2 infection, such as an upregulation in the expression of ACE2, which is not observed with other respiratory viruses. ACE2 is the cellular receptor for the SARS-CoV-2 virus, and its expression along with other certain interferons and cytokines, has been correlated with higher viral doses. Interestingly, down-regulated expression was observed in other gene clusters for olfactory receptor pathways (consistent with the anosmia phenotype in some COVID-19 subjects), lung cell growth, and hematological regulation (Butler et al., 2020). Lastly, additional analysis of this patient dataset based on the altered ACE2 expression findings helped identify high-risk populations and determined that individuals taking ACE pathway inhibitors and angiotensin receptor blockers may have increased susceptibility to SARS-CoV-2 infection (Butler et al., 2020).

Understanding Immune Response

Cell Isolations & Dissociations

Flow Cytometry

In Vitro Cell Culture & Analysis

More information can be extracted by combining sequencing techniques with the biospecimen sample sets. Whole blood from COVID-19 patients can be collected and processed to PBMCs within a day and immunophenotyped using flow cytometry or cultured with new therapeutic agents. Sequencing of the cells can identify drug- or viral-induced changes in these immune cells, further characterizing host response to both the disease and therapy. Whole blood samples collected during routine patient care in acute and convalescent phases, along with PBMCs, have been used to enrich our understanding of the adaptive immune response and how immunity develops to SARS-CoV-2 (Nolan et al., 2020). Similarly, T-cell responses from COVID-19 subject samples have helped characterize the adaptive immune response after recent viral antigenic exposure prior to the ability to detect antibodies and later in the disease course (Snyder et al., 2020). Furthermore, PBMCs from COVID-19 patients have been used to investigate the COVID-19 reactive T cell antigen sequences, which enriches our knowledge of cellular and humoral immune responses to viral infection and our understanding of how active immunization could be modulated (Knierman et al., 2020). This information can be extremely useful for developing new interventions, diagnostics, and monitoring of COVID-19 infections.

Large-Scale Viral Detection and Surveillance

High-quality, scalable, and standardized testing for SARS-CoV-2 is critical as there is a wide range of performance among the Emergency Use Authorization (EUA) SARS-CoV-2 virology tests which may increase false negatives (Arnaout et al., 2020). Differences in key attributes of many tests, such as primer sequences, protocol steps or viral gene targets, and a lack of comprehensive benchmarking across clinical settings with consistent sample types, has made it difficult to compare results or understand the impact on COVID-19 clinical outcomes (MacKay et al., 2020). To this end, Discovery has supported the COVID-19 XPRIZE, OpenCovidScreen, by assisting with proficiency testing development and serving as the validation lab. The COVID-19 XPRIZE aims to identify economically viable and scalable testing options and promote their development, as a step towards enabling truly global viral testing and surveillance (MacKay et al., 2020).

Conclusion

In conclusion, Discovery can offer a comprehensive set of services to support SARS-CoV-2 research. With the world's largest biospecimen inventory and procurement network, combined with preeminent multi-omic service laboratories, Discovery can accelerate precision medicine programs aimed at new therapies, diagnostics, and vaccines. These services have never been more essential, as the rapid pace of science investigating the SARS-CoV-2 virus and COVID-19 requires high-quality research samples and comprehensive sequencing and bioinformatics datasets.

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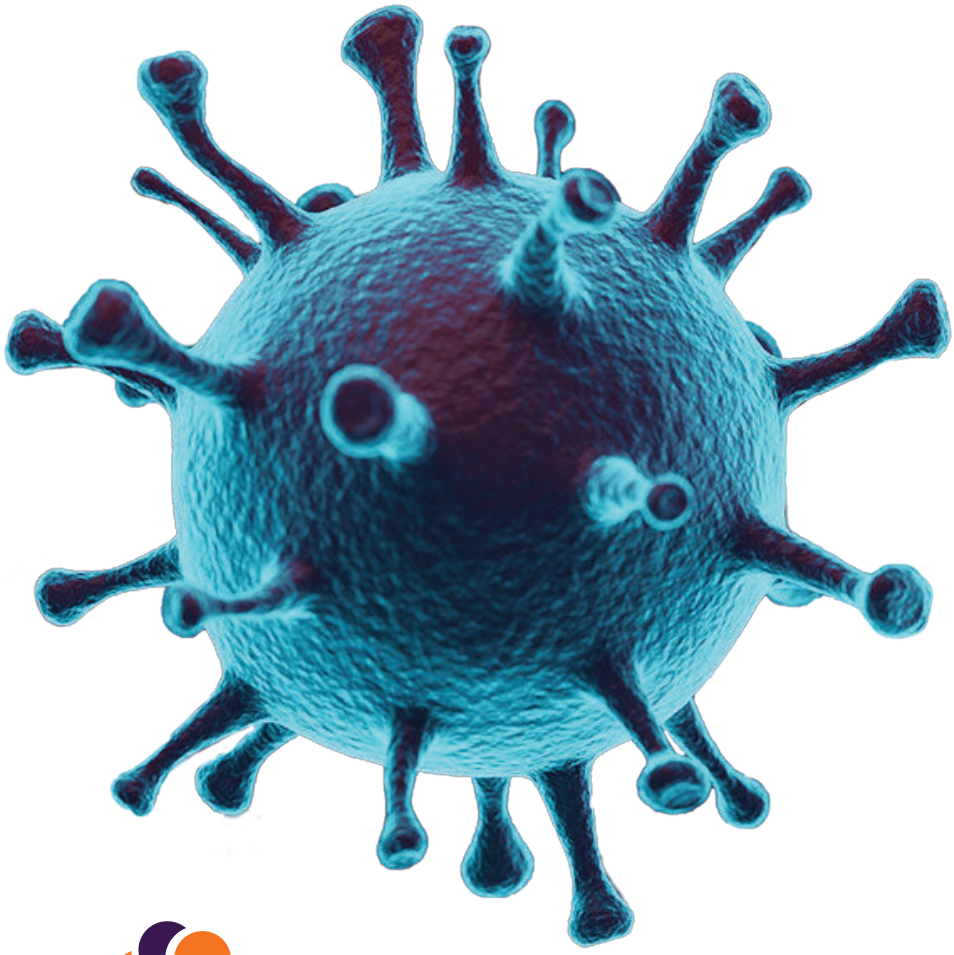

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