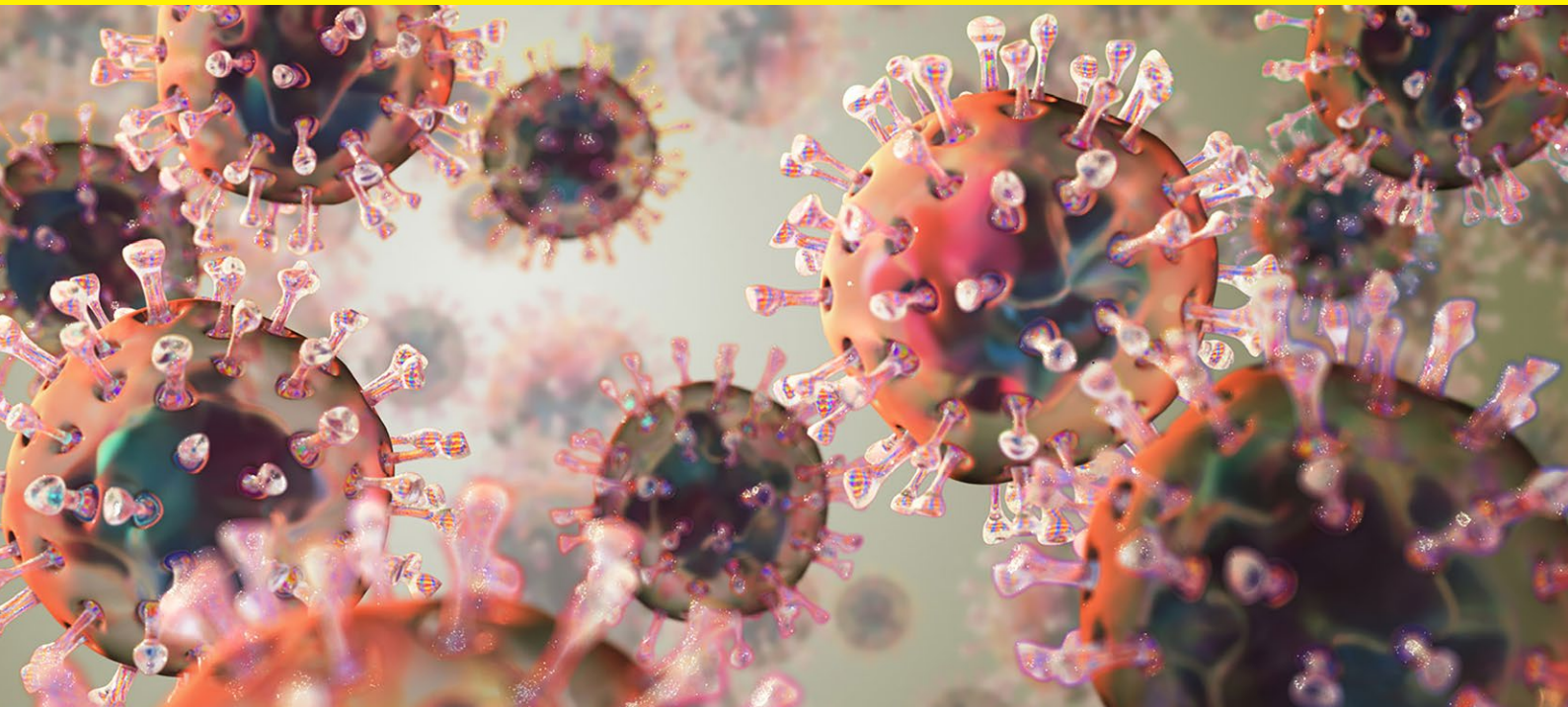
SARTORIUS

Publication Summary: Sartorius Presents Recent Developments in Covid Virology

Articles Included:



Immunology

Multi-Clonal SARS-CoV-2 Neutralization by Antibodies Isolated From Severe COVID-19 Convalescent Donors

Antibodies From Severe SARS-CoV-2 Patients Have Unique B-Cell Receptor (BCR) Signatures Compared to Those From Patients With Mild Disease

COVID-19 is caused by the SARS-CoV-2 virus. While some patients have mild or asymptomatic illness, for others it can be fatal. Understanding the immune response to SARS-CoV-2 at a molecular level is useful for the development of effective vaccines, treatments, and prognostic tests.

SARS-CoV-2 enters the cells through ACE2 (angiotensin-converting enzyme 2) using the receptor binding domain (RBD) of its spike protein. Generation of effective neutralizing antibodies against the RBD is considered critical for controlling SARS-CoV-2. Mor *et al.* (2021) compared BCR signatures of convalescent plasma from eight severe and ten mild cases of SARS-CoV-2 infection taken 1.5 months post infection. Key findings include:

- Convalescent plasma from severe but not mild cases had higher levels of anti-SARS-CoV-2 receptor binding domain (RBD) plasma and IgG titers and increased B cell expansion.
- BCR sequencing revealed that severe SARS-CoV-2 infection was associated with unique B cell signatures, significant enrichment of the VH3-53 gene, and greater B cell clonal expansion.
- Of 22 monoclonal antibodies isolated and cloned from two severe SARS-CoV-2 donors, six were highly potent in neutralizing the live virus and inhibiting the fusion of infected cells.
- Combinations of neutralizing antibodies were more effective than single neutralizing antibodies.
- Antibody neutralization was measured by incubating GFP-labeled pseudovirus with HEK-293 cells stably expressing ACE2 in the presence of serial dilutions of antibodies. The Incucyte® system was used to measure the number of GFP-positive (pseudo-infected) cells.
- To study the potential protective effect of antibodies on SARS-CoV-2-triggered cell death, the team monitored cell viability by staining Vero E6 cells with propidium iodide and measuring them every six hours using an Incucyte® S3 Live-Cell Analysis System.
- To study cell to cell fusion (syncytia formation), the team transfected HEK-293T cells with DNA corresponding to the SARS-CoV-2 spike protein with a 19 amino acid truncation. Target cells were overlaid with untransfected cells in the presence of different antibodies. Cell fusion was measured every 2 hours using the Incucyte® ZOOM system.

Read the full paper in PLOS Pathogens, February 2021

Proteomics, Immunology, Virology

Multilevel Proteomics Reveal Host Perturbations by SARS-CoV-2 and SARS-CoV

Understanding the Function of Viral Proteins and Their Interactions With the Host Proteome Can Lead to the Development of Novel Treatment Strategies

SARS-CoV-2 and SARS-CoV are both members of the coronavirus family that can cause fatal infections in humans. Specific treatments targeting these diseases are limited. To better understand these viruses, many omics studies have been performed. Combining data from different studies can be challenging due to differences in experimental systems.

To obtain a complete data set that can be shared globally, Stukalov *et al.* (2021) performed multi-omics studies in which A549 lung carcinoma cells were transduced with lentiviruses expressing individual HA-tagged viral proteins. This was followed by affinity purification and mass spectroscopy to identify interacting proteins. To study these activities in the context of a viral infection, the team infected ACE2 expressing A549 cells with SARS-CoV-2 or SARS-CoV. The impact of viral infection on mRNA expression (transcriptome), protein abundance (proteome), ubiquitination (ubiquitinome), and phosphorylation (phosphoproteome) were also profiled. Key findings include:

- Comparison of the viral protein-host protein interactions (effectomes) revealed 293 interactions that were unique to SARS-CoV-2, 169 unique to SARS-CoV, and 853 that were shared.
- The transforming growth factor beta (TGF-b) was dysregulated by SARS-CoV-2 but not by SARS-CoV.
- Of 16,541 detected ubiquitination sites, 1,108 were differentially regulated by SARS-CoV or SARS-CoV-2. SARS-CoV-2 specifically increased the ubiquitination of autophagy-related factors.
- Phosphorylation was also impacted by viral infection with 4,643 changed of the 16,399 detected.
- The majority of viral proteins had post-translational modifications.
- Based on the rich data, well characterized drugs were tested as antiviral therapies. A549-ACE2 cells were incubated with the test compound or control, then mock infected with SARS-CoV-2-GFP. The Incucyte® S3 Live-Cell Analysis System was used to capture real-time images of GFP labeled cells every four hours.
- The highest antiviral activity was observed for Gilteritinib (a designated FLT3/AXL inhibitor), Ipatasertib (AKT inhibitor), Prinomastat, and Marimastat (matrix metalloproteases (MMPs) inhibitors).
- MMP inhibitors were selectively inhibited by SARS-CoV-2 activity compared to SARS-CoV.
- This study provides a rich data set for guiding the development of treatments and understanding the mechanisms of SARS-CoV-2 and SARS-CoV infection.

Read the full paper in Nature, April 2021

Immunology

SARS-CoV-2 Specific Antibody & Neutralization Assays Reveal the Wide Range of the Humoral Immune Response to Virus

Development of Highly Sensitive Assays to Detect Antibodies and Their Ability to Neutralize Virus

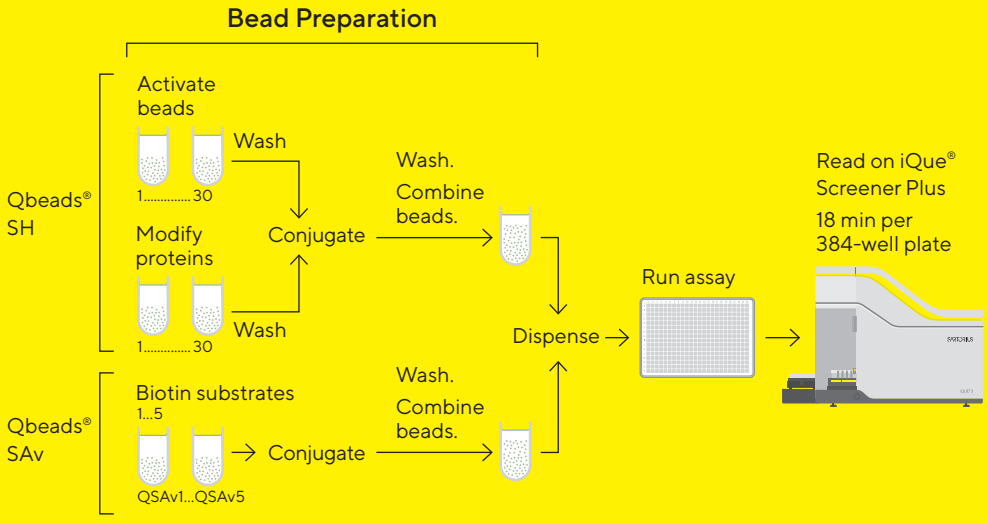
The SARS-CoV-2 virus enters cells though angiotensin converting enzyme-2 (ACE2) using the spike protein. Antibodies against the spike protein have been shown to prevent entry of the virus into the cell neutralizing it. The spike protein is also the basis for many vaccines. Critical tools for studying the immune response to SARS-CoV-2 and vaccines against it are specific antibody tests with a wide dynamic range and neutralization assays.

Dogan *et al.* (2021) developed a highly sensitive bead-based flow cytometry assay. Since this assay can be multiplexed, antibodies to different SARS-CoV-2 proteins and different isotypes can be measured simultaneously. To access antibody neutralization, the team developed a pseudotyped SARS-CoV-2 and SARS-CoV infection assay. These assays were tested using samples from normal donors and the following SARS-CoV-2 positive groups: outpatient, hospitalized, and ICU | diseased patients. Key findings include:

- Antibodies to the SARS-CoV-2 spike protein or nucleocapsid protein were detectable in all SARS-CoV-2 patient groups and were negative in the control group.
- The assay was sensitive to the picogram range and results correlated well with ELISA.
- There were significant differences in levels of antibodies to the receptor binding domain (RBD) of the spike protein and the nucleocapsid protein between outpatient, hospitalized, and ICU | diseased patients. The highest levels were found in the most severe cases.
- Samples from subjects that recovered, potential donors for convalescent plasma, had significantly lower antibody titers than hospitalized, ICU, or diseased patients.
- The iQue Qbeads® DevScreen SAv bead kit was used to bind biotinylated spike protein RBD, nucleocapsid protein, S1 protein, and other proteins to beads for use in fluorescent assays.
- The iQue® Screener Plus was used to measure binding of antibodies to beads labeled with different SARS-CoV-2 proteins. iQue Qbeads® DevScreen SAv beads were gated using FSC-H/SSC-H, and singlet beads gate was created using FSC-A/FSC-H. Gates for different iQue Qbeads® DevScreen SAv beads were determined based on their fluorescence signature on RL1-H/RL2-H plot (on iQue® Screener Plus). PE fluorescence median, which is directly associated with each single plex beads, was determined using BL2-H (on iQue® Screener Plus).
- Entry of both SARS-CoV-2 and SARS-CoV pseudotyped lentivirus into ACE2 expressing cells could be blocked by soluble ACE2. However, there was slightly better blocking of SARS-CoV-2.
- Hospitalized patients had significantly higher neutralization titers compared to outpatients or convalescent donors.

Featured Product

iQue Qbeads® DevScreen SAv, part of the Qbeads® DevScreen family, are bead-based kits that provide you more flexibility to make your own bead-based multiplex assays. iQue Qbeads® DevScreen SAv are streptavidin coated beads that can be used to screen with biotinylated targets. There are 5 different SAv-coated bead populations, and these can be multiplexed.



Read the full paper in Communications Biology, January 2021

Immunology, Biochemistry

The ACE2-Binding Interface of SARS-CoV-2 Spike Inherently Deflects Immune Recognition

Development of a Screening Method to Study the Immunogenicity of Sars-Cov-2 Spike Protein

The spike protein of the SARS-CoV-2 virus is a glycoprotein trimer composed of two subunits, S1 and S2. SARS-CoV-2 enters host cells using the spike protein through the angiotensin converting enzyme-2 (ACE2). A detailed understanding of the receptor binding domain (RBD) of the spike protein and how it interacts with ACE2 is important for the development of effective treatments and vaccines against SARS-CoV-2.

Hattori *et al.* (2021) developed a system to study the binding of antibodies to the ACE2-interacting surface (ACE2IS) located within the receptor-binding domain (RBD). The team created a mutated version of the receptor binding domain (RBD-T) that abolished binding to ACE2. Studies were performed using synthetic human antibodies screened from a library and convalescent sera. Key findings include:

- Patient antibodies predominantly bound to regions of the SARS-CoV-2 spike protein outside the RBD.
- The RBD region of the spike protein was not very immunogenic. Other regions on the spike protein had greater immunogenicity.
- To better understand the immunogenicity of different regions of the spike protein, the team performed *in vitro* antibody selections for the spike and the RBD proteins using biased and unbiased approaches. Unbiased approaches typically led to antibodies outside the ACE2IS while those biased for such regions led to successful enrichment.
- The iQue® screening system was used to detect binding of His-Tagged and biotinylated RBD or the mutated RBD to A549 cells expressing ACE2. Bound RBD or mutated RBD was detected with labeled streptavidin.
- To determine binding of native antibodies or synthetic cloned antibodies to wild type or mutated RBD, biotinylated RBD proteins were bound to streptavidin magnetic beads. They were then incubated with the antibodies, washed, and incubated with a labeled anti-human secondary antibody. The iQue® screen system was used for detection.

Read the full paper in the Journal of Molecular Biology, February 2021

Discover solutions for your Covid research as well as a variety of virology assays here: www.sartorius.com/virology

Germany
Sartorius Lab Instruments
GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 631 254 4249
Toll-free +1 800 635 2906

For further information,
visit www.sartorius.com

Version 1 | 05 | 2021