COVID-19 antibody responses are complex. Profiling them shouldn’t be.

Detect N, RBD, S1, and S2 antibodies simultaneously

Profile the humoral response to COVID-19 infection or vaccination with Bio-Plex Pro SARS-CoV-2 Serology Assays. Choose from IgG, IgM, or IgA-specific panels for highly sensitive and specific detection of antibodies against SARS-CoV-2 nucleocapsid protein and spike protein subunits (receptor binding domain, spike 1, and spike 2). For research use only. Not for use in diagnostic procedures.

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Tracking COVID-19 spread in real time is critical to stop the current global pandemic. Rapid detection is necessary because COVID-19 symptoms, such as cough, fever, and fatigue, are common to other viral diseases, including influenza, so differentiating healthy individuals and patients with other respiratory illnesses from COVID-19 cases is vital.¹

**Identifying SARS-CoV-2 Infection**

Scientists use real-time quantitative PCR (RT-qPCR) to detect SARS-CoV-2; however, RT-qPCR testing produces a significant number of false negatives because the test has limited sensitivity to detect low virus concentrations.

Serological methods offer powerful alternatives to RT-qPCR for detecting infected individuals. With these tests, scientists measure neutralizing antibodies produced by an infected host rather than detecting the virus itself to confirm a viral infection. Several methods test for neutralizing antibodies, such as the plaque reduction neutralization test (PRNT), the pseudotype-based neutralization test (pVNT), and enzyme-linked immunosorbent assay (ELISA).²

**Quantifying Neutralizing Antibodies**

Among the serology methods, the PRNT and pVNT are highly sensitive and specific for measuring antibody levels during viral infections. In PRNT, researchers incubate cells cultured in a dish with serial dilutions of a serum sample and purified virus and then use a microscope to count the number of plaques formed. The serum concentration required to reduce the number of plaques by half compared to a serum-free viral suspension indicates the quantity and effectiveness of antibodies present in the serum.²

Researchers use engineered pseudovirus instead of live virus when performing pVNT. For COVID-19 testing, the pseudovirus carries the genome and the coat spike (S) protein of SARS-CoV-2 enclosed in a capsid from another virus.³ They apply this pseudovirus to engineered cells that overexpress the angiotensin-converting enzyme 2 (ACE2) receptor to facilitate viral entry and reporters such as luciferase. Researchers incubate the pseudovirus and serum with or without antibodies with host cells; a decrease in luciferase signal depicts neutralizing antibodies that inhibit viral entry into cells.

Despite high accuracy and sensitivity, both PRNT and pVNT are unsuitable for COVID-19 surveillance where rapid test results within a few minutes to hours are desirable. The elaborate workflows in PRNT and pVNT take days, making them slow and cumbersome. COVID-19 serological testing needs to be processed in a day, and PRNT and pVNT are not scalable to meet that high demand in clinics.

ELISA offers a sensitive, widely-used alternative for rapidly detecting neutralizing antibodies in infected individuals. In a typical assay, antibody detection occurs directly through antigen-antibody binding in a microtube coated with SARS-CoV-2 antigens. Bioluminescent or fluorescent reporters enable researchers to quantify antibody levels bound to the antigen. Compared to PRNT and pVNT, ELISA workflows are safe, fast, and scalable.⁴ The ability to generate antigen-coated reaction tubes and complete the entire reaction within the single microtube makes ELISA ideal for serology screening.

**Multiplexing With Bio-Plex Immunoassays**

The overall goal of serotesting is to assess infection rates, understand the effectiveness of antibody response, and prevent viral spread. The Bio-Plex Multiplex system is an ELISA-like SARS-CoV-2 serology test for rapidly quantifying a large number of serum samples within a few hours. This easy-to-use microplate assay uses beads coated with multiple SARS-CoV-2-specific antigens such as different spike protein subunits and nucleocapsid in each reaction tube, allowing researchers to measure IgA, IgG, and IgM antibodies against them all in one well. There are a separate set of assays that measure neutralizing antibodies against two wildtype and 11 variant S1, trimeric spike, and RBD proteins in a single well. All-in-one kits include sample preparation and assay reagents to detect SARS-CoV-2-specific antibodies, and a data analysis package automates data processing and visualization. The isotype specific serology and neutralizing assays are sensitive and specific for detecting SARS-CoV-2 antibodies through different stages of infection and among varying disease severity.⁵

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After initial exposure to the virus, the immune system generates highly efficient neutralizing antibodies that persist to protect against future encounters with the virus. Similar to natural infection, vaccination elicits robust immune responses in the form of neutralizing antibodies targeting the injected foreign antigens. Detecting antibodies using serology assays in vaccinated individuals determines the level of immunity, the vaccine efficacy in preventing mild to severe disease in individuals, and the vaccine-induced humoral response protection within a population.

How COVID-19 Vaccines Are Made

The COVID-19 vaccines available today were made using a range of strategies, from well-established approaches to an entirely new method. The most conventional method relies on an inactivated whole virus that generates multiple neutralizing antibodies against different epitopes, as employed in the Sinovac and Sinopharm vaccines. The Oxford/AstraZeneca, Janssen, and Sputnik V vaccines utilized viral capsids from common viruses, such as adenovirus, as vehicles to carry genes that produce SARS-CoV-2-specific antigens to trigger the immune response inside the host.

The Pfizer/BioNTech and Moderna mRNA vaccines deliver mRNA sequences encoding the SARS-CoV-2 spike protein via lipid nanoparticles. The lipid nanoparticle protects the mRNA cargo from degradation and facilitates mRNA sequence insertion into the host cell, while also eliciting an immunogenic response. Once inside the cell, the mRNA is translated into spike proteins that the immune system recognizes and generates antibodies against.

Detecting antibodies using serology assays in vaccinated individuals determines the level of immunity, the vaccine efficacy in preventing mild to severe disease in individuals, and the vaccine-induced humoral response protection within a population.

Seroscreening for Vaccine Epitopes

Identifying highly immunogenic epitopes that elicit robust antibody responses is the first step in epitope-based mRNA and viral-vector vaccine development. In cases where an epitope does not produce a robust immune response, vaccine developers often use adjuvants to boost the immune response and antibody production. Clinical researchers inject the vaccine cargo carrying an epitope with or without an adjuvant into a healthy volunteer to test for the antibody response. Serotesting is critical for measuring the overall antibody response, from screening for immunogenic epitopes in preclinical studies to testing their efficacy in clinical vaccine development.

To hunt for immunogenic SARS-CoV-2 epitopes, researchers used multiple computational and high-throughput screening tools. The viral receptor-binding domain (RBD) in SARS-CoV-2 spike protein interacts with ACE2 receptors on the human nasopharyngeal epithelia during infection. Neutralizing antibodies targeting the RBD and other functional domains in the spike protein are prime candidate epitopes for vaccine development. Spike protein candidate epitopes successfully triggered antibody responses, making them ideal for epitope-based COVID-19 vaccines.

Vaccine testing requires real-time serology monitoring in a large number of participants. Multiplexed, suspension array-based immunoassay platforms enable researchers to screen for epitopes that generate high-titer antibody responses in clinical trials. BioPlex immunoassays enable scientists to detect a broad spectrum of antibody levels after immunization, unraveling how people with different characteristics such as age and physical health respond to vaccines. Following vaccination clinical trials, researchers track the antibody types and molecules involved in inflammation and immunity generated in response to SARS-CoV-2 vaccine candidates to develop safe and effective vaccines.

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SEROTRACKING THE PANDEMIC POST-VACCINATION

From the first reported case of COVID-19 in Wuhan, China to new emerging SARS-CoV-2 variant infections, researchers have characterized immune profiles in affected individuals. The interplay between immune system cells and cytokines is crucial for understanding disease pathophysiology and developing therapeutic strategies. New studies begin to highlight the immune response in individuals receiving vaccines. How long the protective immunity from SARS-CoV-2 lasts, induced by either infection or vaccination, is critical information for clinicians, researchers, and policy-makers. Population serology studies enable researchers to predict how the pandemic will play out.¹

The Hallmarks of Infection and Immunity

The B cells proliferation and antibody production are the body’s adaptive line of defense against foreign agents. Anti-viral Immunoglobulin (Ig) M antibodies are dominant in the early stages of infection. The initial IgM response wanes quickly, and activated B cells secrete IgG and IgA. The magnitude of IgM, IgA, and IgG in the patient samples reflects the time after initial exposure, potential viral load and shedding, and the length of immunity. After viral clearance is achieved, antibody levels begin to decline from the plasma and specialized memory B cells produce low levels of SARS-CoV-2-specific antibodies, providing serological memory months to years after acute infection.²

As with natural infection, serotesting after vaccination reveals the level of immunity in the population, as well as vaccine efficacy. To compare the strength and nature of the immune response after natural infection or vaccination, researchers first measure serum Ig levels. Elevated IgM levels indicate the initial immune response and activation of B cells, whereas an increase in IgG levels suggests long-term immunity. For example, researchers detected peak IgG levels within two weeks in participants receiving the Pfizer/BioNTech vaccine who had previously been infected by SARS-CoV-2. In contrast, those who had not been infected showed a gradual increase in IgG levels that rose rapidly only after the second dose.³ Overall, antibody testing is a powerful tool for navigating the spread of a virus in a population, detecting immune signatures in individuals infected with different variants, and tracking immunity after vaccination.

Beyond measurable serum antibody levels, SARS-CoV-2-specific memory T cell responses are also important correlates of long-term immunity. Specialized CD⁴⁺ memory T cells recognize viral epitopes on the spike protein and nucleoprotein. Memory B and T cells work in concert to generate antibody responses and immunological memory long after the infection is cleared. The B and T cell activation recruits other immune cells such as macrophages and natural killer cells to fight the infection. Researchers recently tested the molecular features of immunosuppression in COVID-19 patients with high viral loads where the patients showed reduced levels of interleukin (IL)-2, tumor necrosis factor (TNF), and lymphotoxin α (LT-α) cytokines and chemokines and high serum IL-1 and IL-6 levels in patients with severe COVID-19.⁴

Longitudinal Serotesting: Immunity and Beyond

Testing serum antibody and immune molecule levels in biological samples from healthy, infected, and vaccinated individuals in longitudinal studies presents a bottleneck in efforts to characterize COVID-19 immune signatures. Advancements in multiplex immunoassay platforms accelerated population serological surveillance programs, which uncovered immune features associated with COVID-19. Bio-Plex multiplex SARS-CoV-2 serology assays are sensitive and specific and they allow researchers to profile immune signatures including IgA, IgG, and IgM antibodies against different SARS-CoV-2-specific antigens. The platform can quantify up to 100 analytes, including cytokines, in a single run, helping researchers determine the association between immune proteins in healthy, vaccinated, and infected individuals. The Bio-Plex Pro™ Human Cytokine 48-Plex Screening panel provides a broader array of cytokine screening kits for studying long-term infection, deleterious inflammation, and immunity characteristics.⁵,⁶,⁷ The antibody measurements guide current policies intending to mitigate disease spread, track emerging variants, achieve herd immunity through mass vaccination, and determine booster dose requirements.

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The Humoral Immune Response During COVID-19 Disease or Post-Vaccination

Natural infection or vaccination confers lasting immunity with SARS-CoV-2-specific IgG and IgM antibodies and the activation of memory B cells. IgM antibodies are dominant in the early stages of infection. After the initial IgM response, activated plasma B cells secrete IgG antibodies. The IgM and IgG antibody levels reflect the time after initial exposure, potential viral load and shedding, and the length of immunity.

Harnessing the Power of Multiplexing

Bio-Plex Pro SARS-CoV-2 neutralization assays quantitate SARS-CoV-2 neutralizing antibodies against two wild type and 11 variants of RBD and spike protein subunits, immune signatures such as cytokines, and IgG, IgA, and IgM antibody classes.
References

1 | Detecting SARS-CoV-2 Neutralizing Antibodies


2 | Winning the COVID-19 Vaccine Race with Multiplex Immunoassays


3 | Serotracking the Pandemic Post-Vaccination


Quickly assay neutralizing antibodies to wild-type and variant SARS-CoV-2 RBD and S1 antigens.

See performance data at bio-rad.com/Bio-PlexSARS-CoV2NAb