Bead-based Multiplex Assay Panels for Quantifying COVID-19 Vaccine-induced Serological Response, Neutralizing Antibodies, and Cytokines

Amy Zhao, Jessica Zhao, Edvelyn Webster, Cassandra Wheat, Binggang Sun, Jessie Ni
BioLegend, San Diego, CA

Abstract

The COVID-19 pandemic, caused by SARS-CoV-2 virus infection, has led to more than 6 million deaths and triggered severe social and economic disruptions worldwide. The emergence of multiple variants of the virus (Alpha, Beta, Gamma, Delta, and Omicron) distinguishes this pandemic as the most dangerous in history. To accelerate COVID-19 research and advance vaccines and treatments, BioLegend developed a series of bead-based multiplex panels including SARS-CoV-2 serological IgA, IgM and IgG, Variants Neutralization Antibody, and COVID-19 Cytokine Storm Panels. These panels simultaneously quantify multiple target analytes in a single biological sample using commonly available flow cytometers. In the representative study described here, we validated these multiplex assay panels using serum and plasma samples obtained from in-house donors before and after first and second vaccination doses. Results clearly show that our multiplex panels serve as powerful tools for precise quantitation of multiple key SARS-CoV-2 analytes in single human biological samples.

Methods

- Flow cytometer capable of detecting PE and APC fluorescence.
- 96-well microtiter filter plates, vacuum pump, filtration manifold, and FACS tubes
- Cloud-based LEGENDplex™ Data Analysis Software Suite

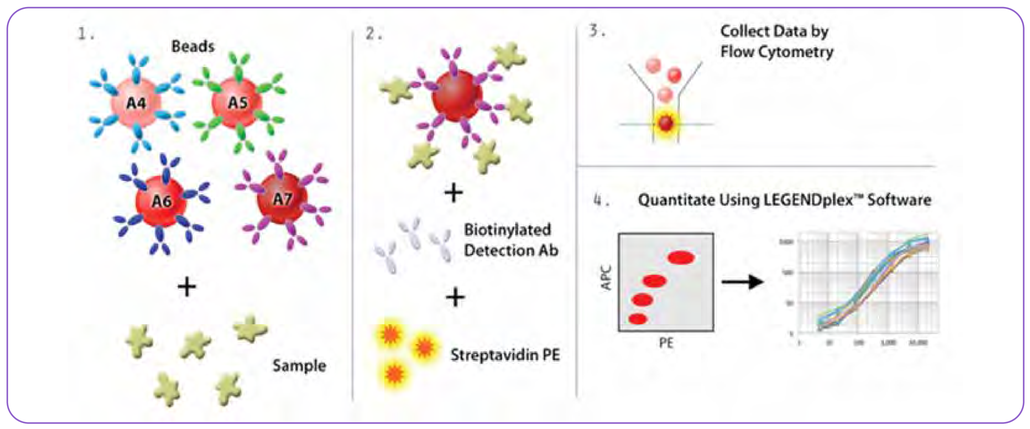
Human serum samples

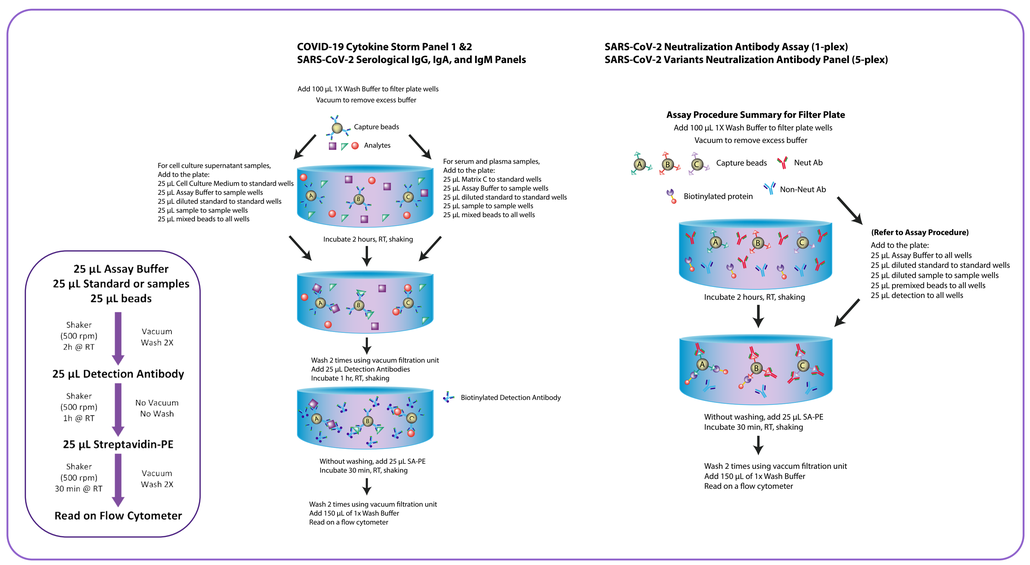
Blood from in-house healthy donors was drawn by venipuncture into Serum Separator Tube (BD, Cat. No. 367820), allowed to clot for 30 minutes, then centrifuged for 10 minutes at 1,000 x *g*. The serum layer was removed and assayed immediately or stored at -20°C and assayed after one thaw. Repeated freeze/thaw cycles should be avoided. Serum specimens should be clear and non-hemolyzed.

LEGENDplex™ Panels

- SARS-CoV-2 Serological IgG Panel (Cat. No. 741131)
  - SARS-CoV-2 S1 Spike Protein, Nucleocapsid, and S Protein RBD
- SARS-CoV-2 Serological IgA Panel (Cat. No. 741138) and IgM Panel (Cat. No. 741207)
  - SARS-CoV-2 S1 Spike Protein and S Protein RBD
- SARS-CoV-2 Neutralization Antibody Assay (1-plex) (Cat. No. 741126)
  - SARS-CoV-2 S1 Variant Wild Type
- SARS-CoV-2 Variants Neutralization Antibody Panel (5-plex) (Cat. No. 741173)
  - SARS-CoV-2 S1 Variant WT, Alpha, Beta, Gamma, and Delta
- COVID-19 Cytokine Storm Panel 1 (14-plex) (Cat. No. 741088)
  - IL-6, MCP-1 (CCL2), G-CSF, IFN-α2, IL-2, IFN-γ, IL-7, IL-1RA, IL-8 (CXCL8), TNF-α, IP-10 (CXCL10), MIP-1α (CCL3), RANTES (CCL5), and IL-10
- COVID-19 Cytokine Storm Panel 2 (13-plex) (Cat. No. 741111)
  - IL-13, GM-CSF, IL-1β, IL-5, sCD25 (IL-2Ra), IL-4, VEGF, IL-17A, IL-18, APRIL, MIP-1β (CCL4), IL-15, and IL-12

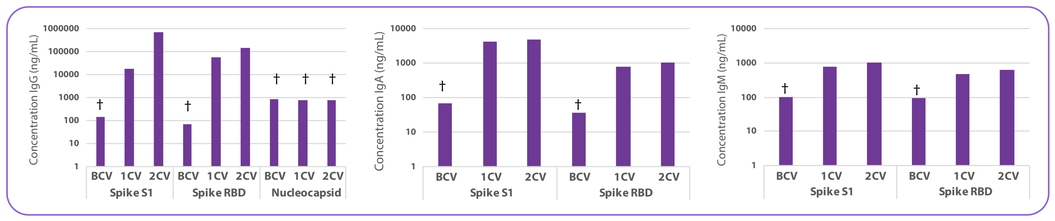
LEGENDplex™ Multi-Analyte Flow Assay Principle





Results

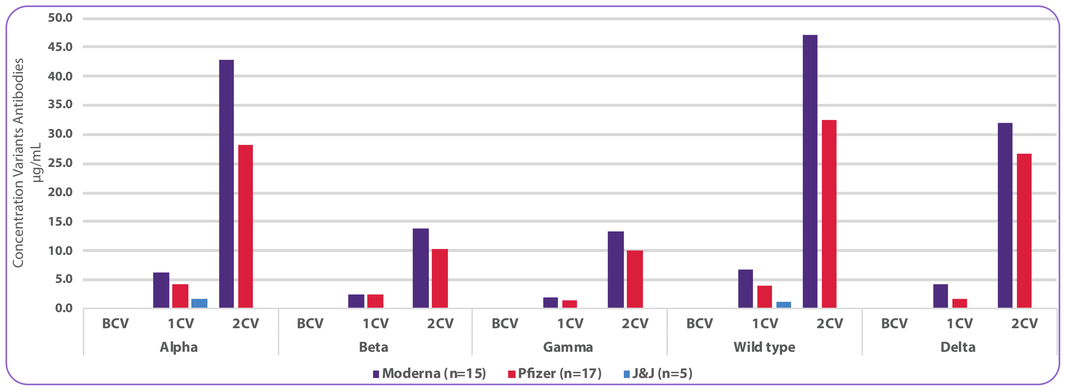
1. SARS-CoV-2 Serological IgG, IgA, and IgM Levels



Serum samples from 32 donors were collected before vaccination (BCV), 2 weeks after the 1st dose of vaccination (1CV), and 2 weeks after the 2nd dose of vaccination (2CV) of the Moderna or Pfizer SARS-CoV-2 vaccines and then tested for IgG, IgA, and IgM concentrations using the appropriate SARS-CoV-2 serological panel.

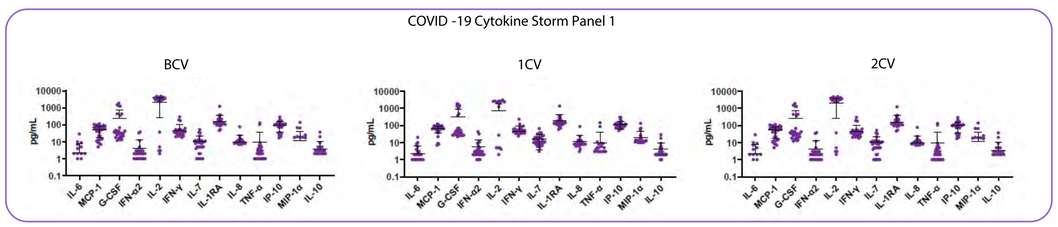
†Predicted concentrations reported between the minimum detectable concentration and C2 (0.488 ng/mL for IgG, 0.976 ng/mL for IgA, or 0.488 ng/mL for IgM) were considered inconclusive data. Pre-existing SARS-CoV-2 reactive antibodies have been reported at low levels in healthy donor cohorts[1]; inconclusive data may reflect this phenomenon and should be verified with clinical information.

2. SARS-CoV-2 Variants Neutralization Antibody Levels

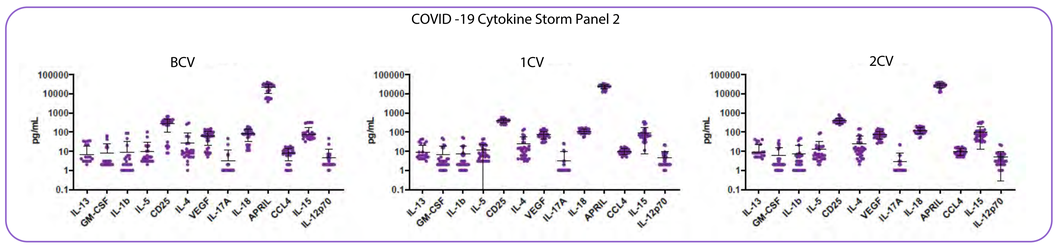


Serum samples from 37 donors were collected before vaccination (BCV), 2 weeks after the 1st dose of vaccination (1CV) (J&J vaccine), and 2 weeks after the 2nd dose of vaccination (2CV) (Moderna or Pfizer SARS-CoV-2 vaccines) then tested for variants neutralization antibody concentrations against SARS-CoV-2 Spike protein Variants Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Wild type S1, and Delta (B.1.617.2) using the SARS-CoV-2 Variants Neutralization Antibody Panel (5-plex).

3. Cytokine Profile in Serum Samples Before and After COVID-19 Vaccine



* Data for cytokine RANTES in the COVID-19 Cytokine Storm Panel 1 are not included here due to its saturation in most of the samples under the same dilution factor.



Serum samples from 32 donors were collected before vaccination (BCV), 2 weeks after the 1st dose of vaccination (1CV), and 2 weeks after the 2nd dose of vaccination (2CV) of the Moderna or Pfizer SARS-CoV-2 vaccines then tested for cytokine concentrations using the appropriate cytokine panel.

Conclusions

- Data from our representative study showed increases in IgG, IgA, and IgM antibody titers and high concentration of neutralization antibodies in blood samples from donors who received COVID-19 vaccinations.
- There was no discernible difference in cytokine profile pre- and post-vaccination. This upholds other reports that COVID-19 vaccines do not put patients at higher risk of developing cytokine storm.
- SARS-CoV-2 LEGENDplex™ Panels have been optimized for assay specificity, sensitivity, accuracy, and reproducibility. Data not shown here but can be found in product manuals.
- SARS-CoV-2 LEGENDplex™ Panels have been validated to detect the target analytes in human biological samples from donors before vaccination and two weeks after the first and the second doses of vaccination.
- Our high-quality, cost-effective, and easy-to-use SARS-CoV-2 LEGENDplex™ Panels are reliable research tools for studying vaccine development and COVID-19 research.

References

1. K.W. Ng *et al.*, *Science*. 370 (2020), doi: 10.1126/science.abe1107