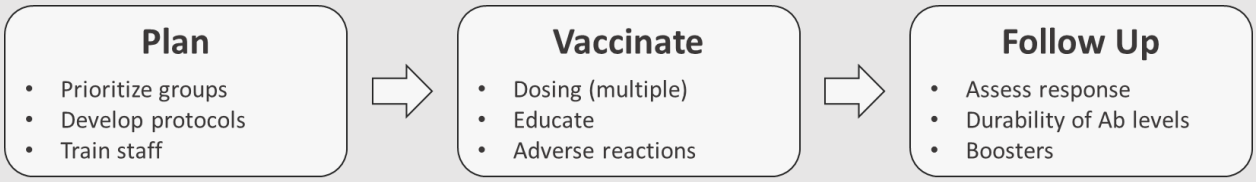
Quantitative serology testing can better assess immune response following SARS-CoV-2 vaccination

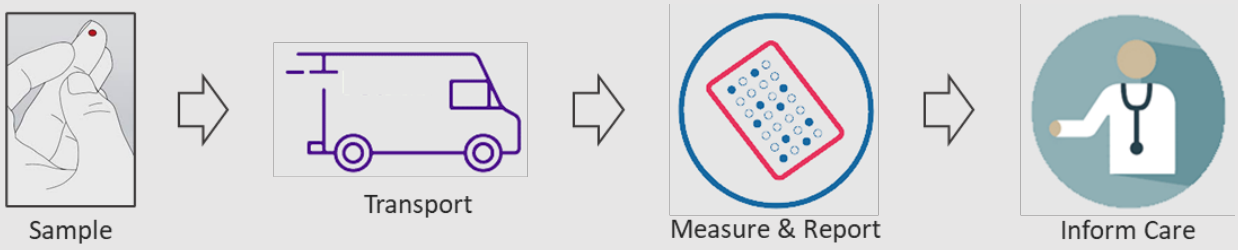
Overview

An important application of COVID-19 serology tests is measuring an individual's immune response after vaccination[1]. The novel nature of SARS-CoV-2 and limited experience with newly developed vaccines motivates the inclusion of immune status monitoring post vaccination. In particular, quantitative serology testing can assess an individual's response after vaccine dosing as well as the longer-term durability of antibody levels.



ProterixBio's quantitative COVID-19 serology test is well-suited for vaccination follow up:

- Measure antibodies that bind the RBD of the spike protein and are associated with neutralizing activity
- Reproducible numerical output allows quantitative assessment: track levels over extended periods
- Validated quantitative performance for a variety of sample types
- Capable of analyzing dried blood spots (DBS) for convenient finger stick sampling
- High-throughput CLIA laboratory testing service, easy workflow and fast turnaround times



Deployment of vaccines

The deployment of the first SARS-CoV-2 vaccines is rapidly expanding to the broader population. This is welcome news for everyone, especially healthcare organizations that have been working to combat the pandemic. While the safety and efficacy data are promising, a lack of experience with SARS-CoV-2 vaccines motivates monitoring after vaccination. There are multiple manufacturers and vaccines, and the durability of the antibody levels generated is not fully understood. Incorporating quantitative serology testing following vaccination enables monitoring of an individual's immune response and durability antibody levels. This is particularly important for healthcare workers, essential workers and at-risk individuals.

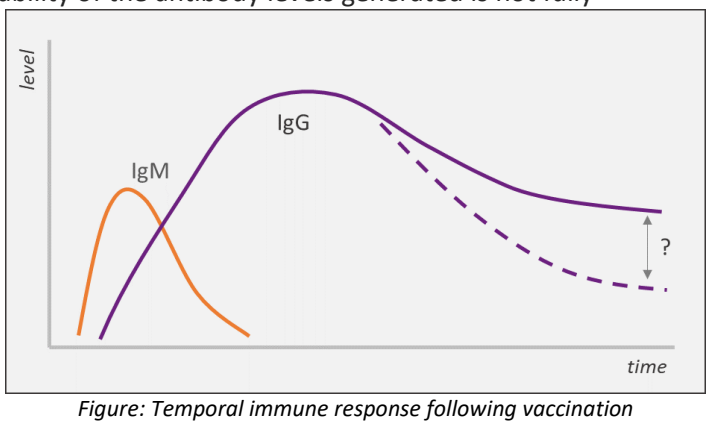


Figure: Temporal immune response following vaccination

Vaccine designs and neutralizing antibodies

There are several types of vaccines being developed, including mRNA, adenovirus and traditional protein.[2] Irrespective of design, all incorporate versions of the spike protein and its Receptor Binding Domain (RBD) as

ProterixBio, Inc. **(P) +1.978.901.6700** info@proterixbio.com
February 2021

the immunogen. The RBD is the region of the spike protein that binds the ACE2 receptor of host cells and is central to COVID-19 pathology.

The goal of a vaccine is to generate a robust immune response that includes durable, protective levels of neutralizing IgG antibodies. Neutralization refers to an antibody's ability to inhibit the virus from infecting a host cell by blocking the binding of the RBD to the ACE2 receptor. Neutralizing antibodies are an important component of protective immunity. Other categories of therapeutics also rely on neutralizing antibodies to confer benefit, including convalescent plasma, monoclonal antibodies and hyperimmune globulin. Serology tests used to monitor individuals post vaccination need to measure antibodies that bind antigens incorporated into vaccines (e.g., RBD) and be correlated with neutralizing activity so the results can be interpreted on a standardized basis.

ProterixBio's quantitative COVID-19 serology test provides unique insights

Many currently-available COVID-19 serology tests are qualitative and do not provide information about the level of antibodies. They provide just a yes/no answer indicating presence or absence. ProterixBio quantitative COVID-19 serology test: 1) detects antibodies that bind the RBD and are associated with neutralizing activity, and 2) provide a quantitative output that is referenced to neutralizing titer for interpretation on a standardized basis and can be tracked over time. ProterixBio also adds the convenience of finger stick sampling by supporting dried blood spot analysis.

Higher levels of IgG antibodies binding the RBD have been associated with higher neutralizing titers in studies of vaccines as well as convalescent plasma.[3-7] ProterixBio has associated the numerical index of its SARS-CoV-2 (RBD) IgG Antibody test with neutralizing activity so the results can be interpreted on the same basis used to support vaccine efficacy. As shown in the Figure, higher test index values are associated with higher levels of neutralizing activity similar to those reported in vaccine trials and that are anticipated in individuals post vaccination.

Vaccine trials have documented antibody levels lasting several months after dosing, although the long-term durability is not yet known. Studies of select populations of convalescent individuals have observed antibody levels persisting for at least 3-6 months but variations have been observed between individuals.[6-8] ProterixBio's COVID-19 serology test provides a means to assess an individual's response a few weeks after dosing as well as monitor the durability of antibody levels over time. This information may allow individuals and their physician to make better decisions regarding their health.

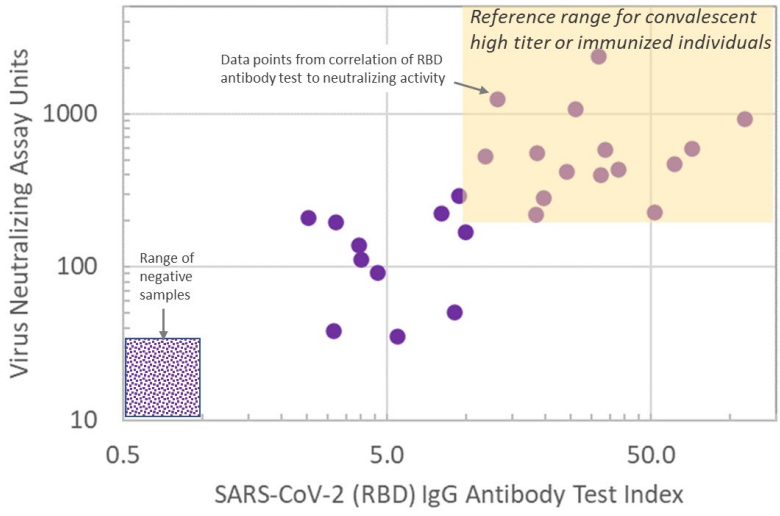


Figure: Example of overlaying reference range with correlation of ProterixBio's quantitative COVID-19 serology test and neutralizing activity (details below).

ProterixBio test performance summary

The sensitivity of the ProterixBio's SARS-COV-2 (RBD) IgG Antibody test was assessed by analyzing blood samples from 70 individuals with prior SARS-CoV-2 infections confirmed by PCR. The Positive Percent Agreement (PPA) was 100% for all samples acquired ≥15 days post onset of symptoms. For samples acquired earlier (0-14 days), the PPA decreased to 50% reflecting variable seroconversion rates among individuals. For individuals sampled >112 days post symptoms onset, the mean index value was lower than that observed at earlier time points; however, all samples remained positive (index >1.0).

The specificity of the SARS-COV-2 (RBD) IgG Antibody test was assessed by analyzing blood samples acquired pre-pandemic. Several populations were assessed including adults covering a range of demographic categories, chronic conditions, exposure to common infectious diseases and vaccination history and children who had been hospitalized for a variety of conditions. The Negative Percent Agreement (NPA) was 98.9% or greater for all groups. The specificity of the assay was further tested with samples with confirmed levels of antibodies to other infectious agents (e.g., HIV-1, HCV, CVM, etc.) which yielded negative results for all samples tested.

Positive Percent Agreement (PPA)

Days after Symptoms Onset	ProterixBio SARS-CoV-2 (RBD) IgG Antibody Test				
	Total	Mean Index*	Positive	Negative	PPA
0 - 14 days	6	2.1	3	3	50%
15 - 28 days	9	10.5	9	0	100%
29 - 56 days	34	13.5	34	0	100%
57 - 112 days	14	25.1	14	0	100%
113 - 165 days	7	9.9	7	0	100%

*Average of sample index values from different individuals

Negative Percent Agreement (NPA)

Pre-2019, U.S. Population Groups	ProterixBio SARS-CoV-2 (RBD) IgG Antibody Test			
	Total	Positive	Negative	NPA
Children (<18 yrs)	100	0	100	100%
Adults (18-80 yrs)	127	0	127	100%
Adults w/ chronic respiratory disease (50-80 yrs)	282	3	279	98.9%

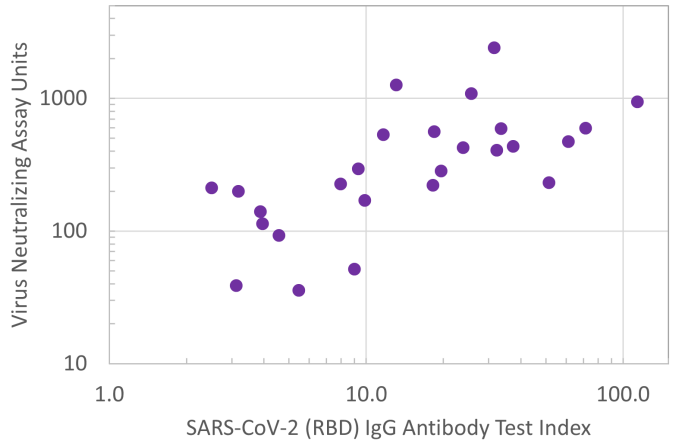
Quantitative Performance

The assay output is a numerical index that is the ratio of the antibody concentration of the sample to that of a calibrator level set at the positive/negative cut-off. The results below demonstrate excellent precision across the assay range. Linearity of dilution was also characterized, and the assay recovers to within 10% of expected for a range of dilutions (data not shown).

Sample I.D.	N	Mean Index	Within Run		Day to Day	
			SD	CV%	SD	CV%
Sample A	80	16.65	1.23	7.1	0.60	3.6
Sample B	80	6.80	0.52	7.3	0.50	7.4
Sample C	80	3.39	0.17	4.7	0.28	8.2
Sample D	80	1.66	0.11	7.1	0.28	16.7
Sample E	80	0.54*	0.05	11.3	0.10	17.8

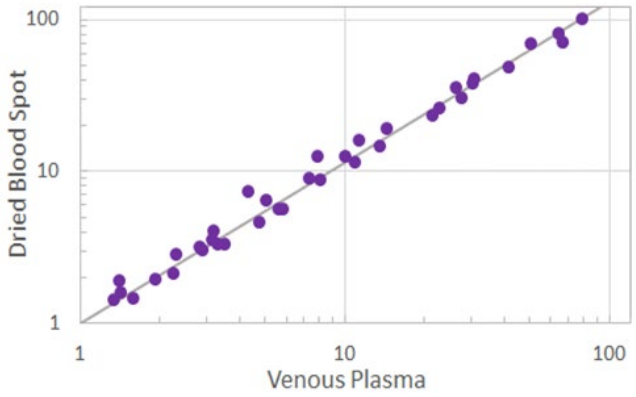
*Measured level is below the pos/neg cut-off (Index = 1) but above LoQ for the assay

Neutralizing Activity



Seropositive samples (N=26), which were all >20 days post symptoms onset, were characterized with a neutralization assay and the ProterixBio (RBD) SARS-CoV-2 IgG Antibody test. The data are associated with Spearman rank-order, correlation coefficient = 0.74 and p value < 0.001. For reference, virus neutralizing assay units of 150 - 300 correspond to a PRNT ID50 = 1:640.

Dried Blood Spot / Finger Stick Performance



Dried blood spots (DBS) from finger sticks are compared to venous EDTA plasma (N=37). Deming regression of ProterixBio SARS-CoV-2 (RBD) IgG Antibody test index values measured in both samples yielded a fit with slope = 1.06, p < 1e-30, and a correlation coefficient = 0.99. These data demonstrate the equivalence of the quantitative test performance in the different sample types.

References

1. Krammer, F. and Simon, V., Serology assays to manage COVID-19, Science, 15May, 2020.
2. Liu et al, SARS-CoV-2 Vaccine Development: An Overview and Perspectives, ACS Pharmacol. Transl. Sci., 3, 844-858, 2020.
3. Jackson et al, An mRNA Vaccine against SARS-CoV-2 – Preliminary Report, NEJM, 14July, 2020.
4. Walsh et al, RNA-Based COVID-19 Vaccine BNT162b2 Selected for a Pivotal Efficacy Study, medRxiv, 20August, 2020.
5. Widge et al, Durability of Responses after SARS-CoV-2 mRNA-1273 Vaccination, NEJM, 6January 2021
6. Wajnberg et al, SARS-CoV-2 infection induces robust, neutralizing antibody responses that are stable for at least three months, medRxiv, 17July, 2020.
7. Seow et al, Longitudinal evaluation and decline of antibody responses in SARS-CoV-2 infection, medRxiv, 11July, 2020
8. Gudbjartsson et al., Humoral Immune Response to SARS-CoV-2 in Iceland, NEJM, 1Sept 2020.

ProterixBio, Inc. **(P) +1.978.901.6700** info@proterixbio.com

February 2021