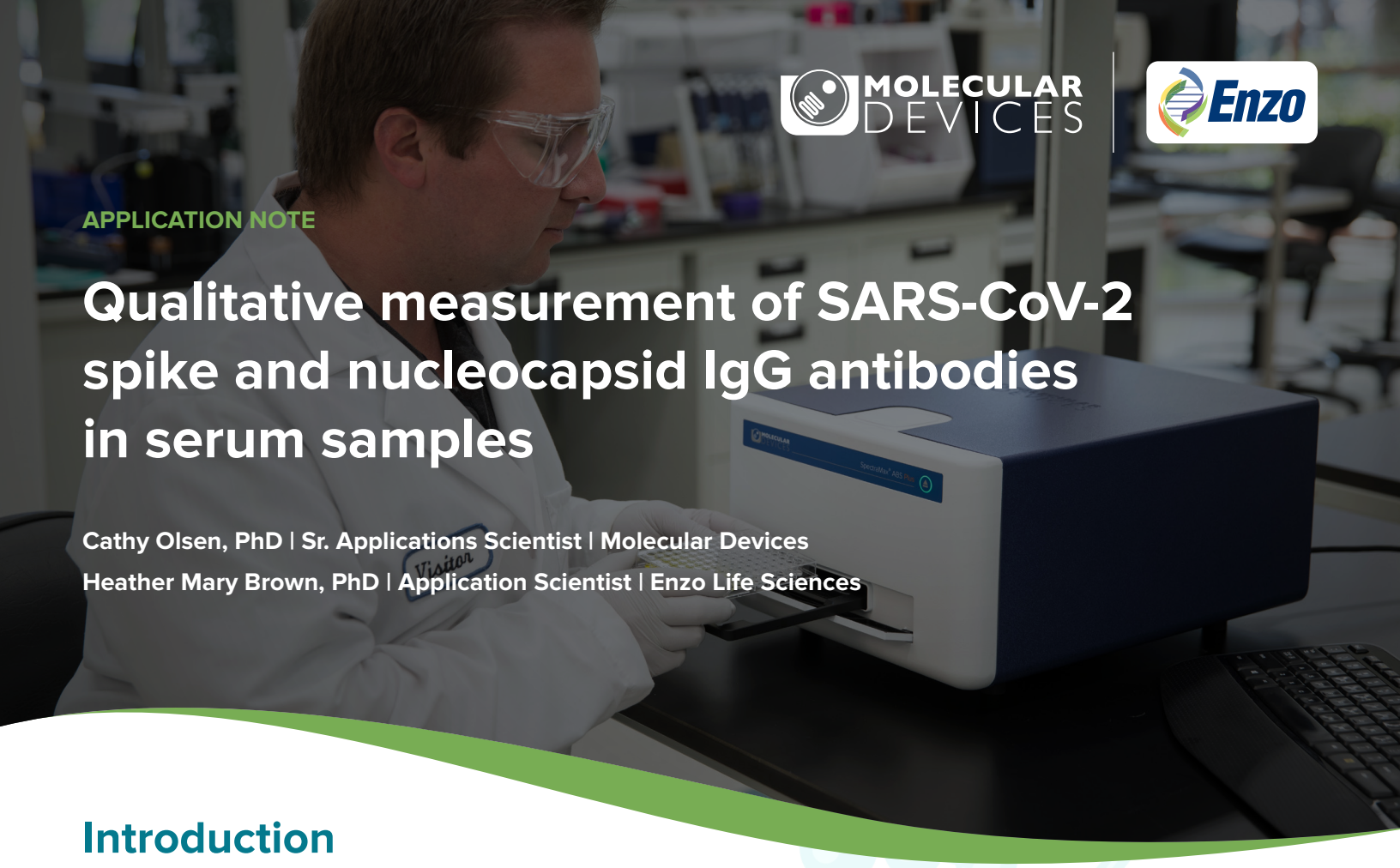

APPLICATION NOTE

Qualitative measurement of SARS-CoV-2 spike and nucleocapsid IgG antibodies in serum samples

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Introduction

Rapid, accurate, and frequent molecular testing is of critical importance in navigating the SARS-CoV-2 (COVID-19) pandemic. To advance our understanding of the relationship between infection and immunity, it is critical to test not only for infection, but also for antibodies. The fundamental challenge is to identify and implement sensitive, consistent, and accessible testing methods that fulfill these requirements, all at an affordable price. Quantitative RT-PCR testing for detecting viral mRNA is currently the most accurate test on the market for identifying an existing infection. However, it does not shed light on the immune response. Given that significant emphasis has been placed on developing testing methods to identify disease, advancements in assay development for antibody testing have remained insufficient. The antibody tests currently on the market return many false positive results and therefore are not a reliable method for understanding the nuances of the immune response to SARS-CoV-2[1]. Thus, a reliable, high-throughput method for investigating COVID-19 immunity and antibody response is an unmet need.

Enzo has developed a state-of-the-art enzyme linked immunosorbent assay (ELISA) that is specifically optimized to detect IgG antibodies against the SARS-CoV-2 Nucleocapsid protein in human serum and plasma samples (ENZ-KIT193). SARS-CoV-2 Nucleocapsid IgG protein is an antibody generated as part of the adaptive human immune response to the Nucleocapsid protein of the SARS-CoV-2 virus. The Nucleocapsid protein is important for RNA packaging and virus particle release. The presence of antibodies against the Nucleocapsid protein indicate a recent or prior infection. The Nucleocapsid protein is highly conserved and is less susceptible to mutations over time, making this an optimal target for long-term studies of COVID-19 immunity[2].

Additionally, Enzo has developed a complimentary ELISA kit, SARS-CoV-2 Spike IgG ELISA Kit (RUO) (ENZ-KIT190), enabling detection of specific antibodies against the SARS-CoV-2 Spike protein in human serum. SARS-CoV-2 Spike IgG protein is another antibody generated via the adaptive human immune response to the Spike protein of the SARS-CoV-2 virus. Spike protein is essential for binding of the virus to the ACE2 receptor on the surface of human cells for entry into the cell for replication. Thus, it is a target for some vaccines currently available.

Benefits

- Gain >95% specificity for nucleocapsid and spike IgG antibodies
- Measure up to 86 samples per kit with high-throughput, qualitative immunoassays
- Acquire colorimetric readout with exceptional inter/intra assay precision

Enzo's SARS-CoV-2 Nucleocapsid IgG ELISA Kit and SARS-CoV-2 Spike IgG ELISA Kit are keystone tools for current and future studies to determine antibody response and immunity to COVID-19 infection and vaccine studies.

Both ELISA kits exhibit a broad detection range, high sensitivity, and >95% specificity for their target analytes, Nucleocapsid IgG antibody and Spike IgG antibody, in up to 86 samples within 30 minutes or two hours, respectively. Thus, these kits exceed requirements for accuracy and speed, and offer the potential for many applications to propel research forward. Developing a deeper understanding of the antibody behavior post-virus exposure will allow researchers to reveal characteristics of SARS-CoV-2 immune response following infection, and also serve as a tool for understanding the fundamental questions regarding immunity to this highly infectious virus.

Materials

- SARS-CoV-2 Spike IgG ELISA Kit (RUO) (Enzo cat. #ENZ-KIT190-0001)
- SARS-CoV-2 Nucleocapsid IgG ELISA Kit (RUO) (Enzo cat. #ENZ-KIT193-0001)
- Human Serum COVID-19 Positive (BioIVT cat. #HMSRM-COVIDIGG):
  - Lot #HMN368394-SR1
  - Lot #HMN368436-SR1
  - Lot #HMN368437-SR1
  - Lot #HMN373782-SR1
  - Lot #HMN373791-SR1
  - Lot #HMN374206-SR1
- Human serum, off the clot (negative) (Amsbio cat. #HSER-2mL):
  - Lot #122019A
- SpectraMax® ABS Plus Microplate Reader

Methods

Assay setup

Materials from each of the two kits were brought to room temperature prior to use, including well strips sufficient to run assay controls and duplicate serum samples for each kit. 1X Wash Buffer was prepared by diluting the 20X Wash Buffer Concentrate with water.

Serum samples from patients who had tested positive for SARS-CoV-2, or normal serum collected pre-pandemic, were prepared by diluting them in Sample Diluent as indicated in the product manual for each kit.

The steps for running each ELISA kit are shown in Table 1.

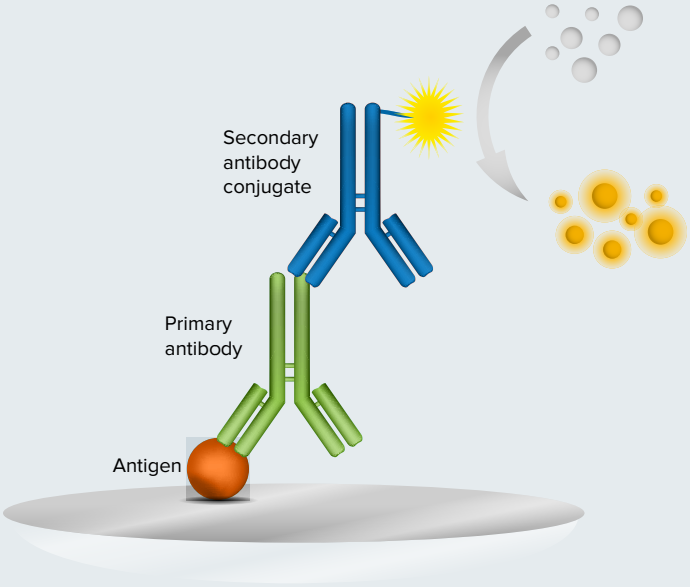

Figure 1. *SARS-CoV-2 IgG ELISA (indirect ELISA format). SARS-CoV-2 IgG ELISAs schematic. The bottom of the wells are coated with SARS-CoV-2 Nucleocapsid protein or Spike S1-receptor binding domain (antigen). If present in the serum sample, IgG specific for the antigen binds to the coated wells, and an HRP-conjugated antibody enables detection of the bound IgG with the addition of substrate.*

SARS-CoV-2 Nucleocapsid IgG ELISA Kit	SARS-CoV-2 Spike IgG ELISA Kit
Dilute serum samples 1:200 with Sample Diluent	Dilute serum samples 1:10 with Sample Diluent
Add Calibrators and samples to wells	Add High, Low, Negative Controls and samples to wells
Incubate 10 minutes at room temperature (RT)	Incubate for 30 minutes at 37°C
Wash 3X with Wash Buffer	Wash 4X with Wash Buffer
Add Nucleocapsid Conjugate to wells	Add HRP Conjugate to wells
Incubate 10 minutes	Incubate for 15 minutes at 37°C
Wash 3X with Wash Buffer	Wash 5X with Wash Buffer
Add TMB Substrate to all wells	Add TMB Substrate to all wells
Incubate 10 minutes at RT	Incubate for 15 minutes at 37°C in the dark
Add Stop Solution	Add Stop Solution
Read plate at 450 nm	Read plate at 450 nm

Table 1. *Steps for running each SARS-CoV-2 ELISA Kit.*

Calculation of results for SARS-CoV-2 Nucleocapsid IgG ELISA

Net OD values were calculated by subtracting the average OD value of the blank wells from all OD values for all wells, including the calibrators and serum samples.

Net Sample OD = Sample OD - Average Blank OD

The average OD value for each calibrator was plotted vs. its concentration using a 4-parameter logistic curve fit in SoftMax® Pro Software (Figure 2).

Results were then calculated semi-quantitatively by interpolation off the calibrator curve. An interpolated concentration ≤ 2000 ng/mL implied the absence of SARS-CoV-2 IgG, while an interpolated concentration > 2000 ng/mL implied the presence of SARS-CoV-2 IgG.

Calculation of results for SARS-CoV-2 Spike IgG ELISA

Net OD values were calculated by subtracting the average OD value of the blank wells from all OD values for all wells, including the Negative Control, Low Positive Control, High Positive Control, and serum samples:

Net Sample OD = Sample OD - Average Blank OD

An Index Value was calculated for each sample using the following formula:

$$\textit{Index Value} = \frac{\textit{Net Sample OD}}{\textit{Cutoff Value (CoV)}}$$

A cut-off value of 0.08 was given in the product manual and used to calculate results. Index values ≥ 1 indicated a positive result, while index values < 1 indicated a negative result. All calculations were set up in group tables in SoftMax Pro Software, enabling automatic calculation of results when the ELISA plate was read.

Results

For both ELISA kits, the serum samples from COVID-19 positive patients tested positive, meaning that all of these samples contained both nucleocapsid IgG and spike IgG. The normal serum sample tested negative for both assays. Group tables from SoftMax Pro Software showing results automatically calculated from the data are shown in Table 2 (Nucleocapsid IgG ELISA kit) and Table 3 (Spike IgG ELISA kit).

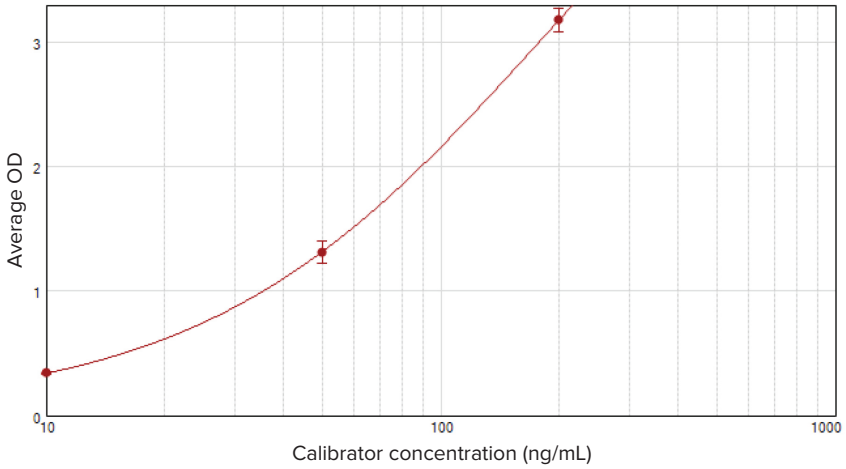

Figure 2. *Calibrator curve for the SARS-CoV-2 Nucleocapsid IgG ELISA Kit. The curve was plotted in SoftMax Pro Software using a 4-parameter logistic curve fit. From this curve, results were calculated semi-quantitatively.*

Sample	Wells	Value	AvgValue	R	Result	MeanResult	SD	%CV	Dilution	AdjResult_ng/mL	SemiQuantResult
01	C2	0.401	0.365		12.201	10.923	1.807	16.5	200	2184.545	POS
	D2	0.328			9.645						
02	E2	3.449	3.469	R	239.379	242.624	4.590	1.9	200	48524.844	POS
	F2	3.488		R	245.870						
03	G2	2.192	2.092		102.831	95.781	9.969	10.4	200	19156.222	POS
	H2	1.991			88.732						
04	A3	2.144	2.205		99.288	103.809	6.394	6.2	200	20761.716	POS
	B3	2.266			108.329						
05	C3	2.160	2.155		100.476	100.090	0.546	0.5	200	20017.992	POS
	D3	2.149			99.704						
06	E3	0.594	0.571		19.202	18.331	1.231	6.7	200	3666.248	POS
	F3	0.547			17.461						
07	G3	0.066	0.061		0.923	0.743	0.254	34.2	200	148.685	NEG
	H3	0.055			0.564						

Table 2. *Results of SARS-CoV-2 Nucleocapsid IgG ELISA Kit. Results were calculated semi-quantitatively via interpolation from the calibrator curve and displayed in a group table in SoftMax Pro Software. 'R' indicates samples with OD values that fell outside the range of the calibrator curve.*

Sample	Well	NetValues	MeanNetValue	Std.Dev.	CV%	IndexValue	Result
01	C2	1.771	1.777	0.009	0.497	22.216	POS
	D2	1.784					
02	E2	2.100	2.062	0.054	2.610	25.774	POS
	F2	2.024					
03	G2	2.508	2.463	0.064	2.604	30.788	POS
	H2	2.418					
04	A3	1.972	1.981	0.013	0.650	24.765	POS
	B3	1.990					
05	C3	1.869	1.837	0.046	2.498	22.962	POS
	D3	1.804					
06	E3	0.790	0.778	0.017	2.190	9.727	POS
	F3	0.766					
07	G3	0.039	0.034	0.007	21.822	0.421	NEG
	H3	0.028					

Table 3. *Results of SARS-CoV-2 Spike IgG ELISA Kit. Index values were calculated and sample results were displayed as positive or negative in a group table in SoftMax Pro Software.*

Conclusion

Overall, these data show that the Enzo kits, SARS-CoV-2 Nucleocapsid IgG ELISA Kit (RUO) (ENZ-KIT193) and SARS-CoV-2 Spike IgG ELISA Kit (RUO) (ENZ-KIT190), are an accurate, high-throughput and rapid solution for the detection of Nucleocapsid IgG antibodies and Spike IgG antibodies in human samples. Furthermore, these kits are easily run on the SpectraMax ABS Plus reader with SoftMax Pro Software, which enable consistent and efficient generation of results. These quantitative and semi-quantitative data provide valuable metrics for specific IgG analysis for an individual patient sample as well as accurate data to contribute to population surveillance studies. Thus, they present novel opportunities for implementation of these solutions in the clinic for future investigations into understanding the nuances of the immunogenic response, duration of immunity, and patterns in population studies of infection and immunity response to SARS-CoV-2. The applications for both kits can play a major role in the prevention and management of future pandemics.

References

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2. Dutta NK, Mazumdar K, Gordy JT. The Nucleocapsid Protein of SARSCoV-2: a Target for Vaccine Development. *J Virol.* 2020;94(13):e00647-20. Published 2020 Jun 16. doi:10.1128/JVI.00647-20

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