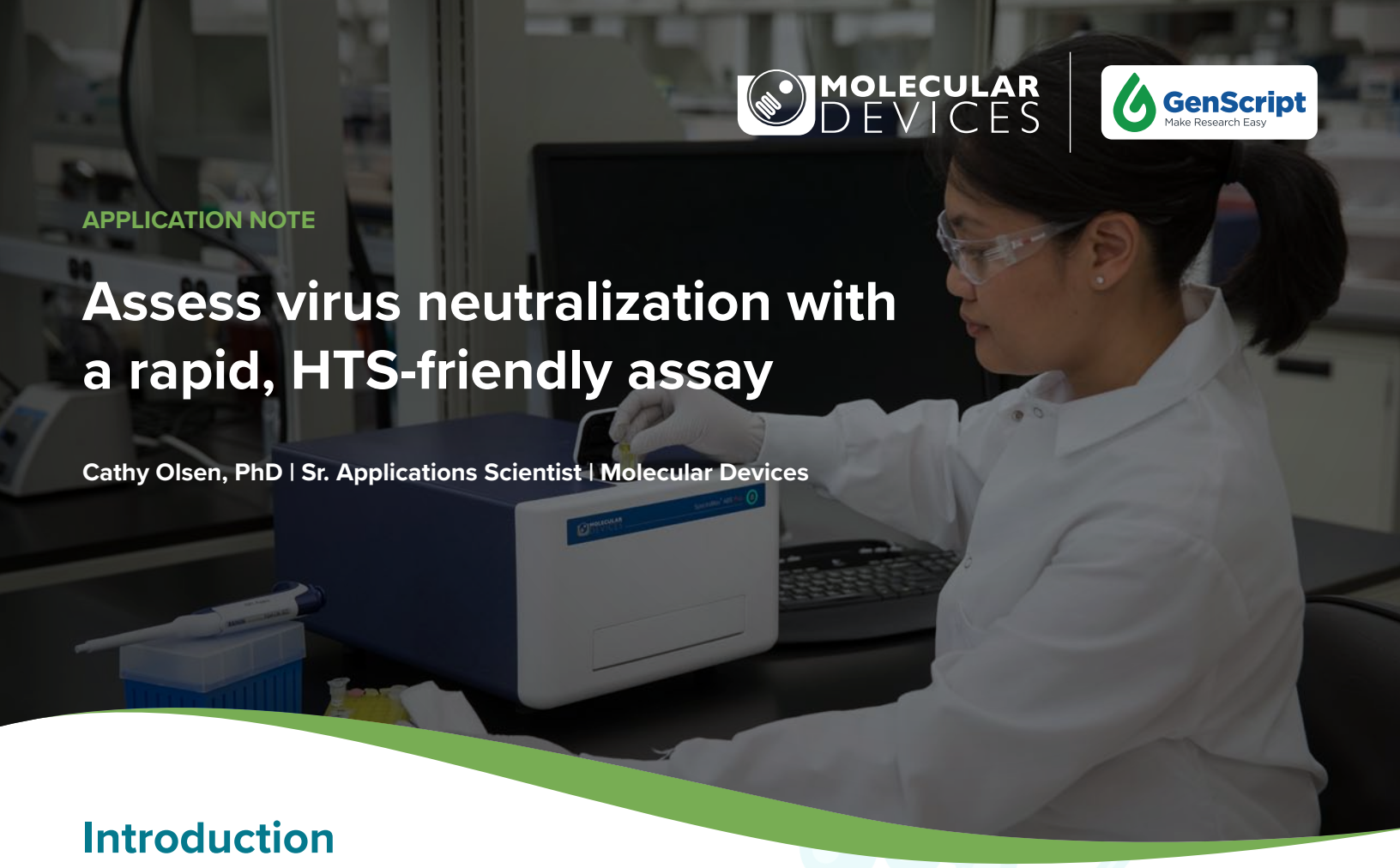

APPLICATION NOTE

Assess virus neutralization with a rapid, HTS-friendly assay

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Introduction

The worldwide COVID-19 pandemic caused by the SARS-CoV-2 virus has necessitated the fast-tracked development of many research tools for understanding this virus's pathogenesis, as well as vaccine discovery and development. Assays for monitoring the immune response to infection by and vaccination against the virus are important to COVID-19 research. As neutralizing antibodies are key biomarkers of immune response and vaccine efficacy, levels of neutralizing antibodies in patient serum samples is an important parameter to be able to monitor efficiently.[1]

Identification of neutralizing antibodies in human serum has commonly been performed using traditional methods like the plaque reduction neutralization (PRNT) assay. However, because PRNT uses live, infectious virus added to target cells, it requires a BSL-3 laboratory and laborious cell culture techniques. Analysis of the resulting data is time consuming and not amenable to automation. Alternatively, the pseudovirus neutralization test (pVNT) method can be done in a BSL-2 lab using a modified form of the virus, but it requires cell culture and fluorescence imaging to measure neutralizing activity, so it is also not suited to high-throughput screening of samples.[2]

The GenScript cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit is a surrogate virus neutralization test (sVNT) developed to overcome the hurdles of more conventional virus neutralization assays. It employs an enzyme-lnked immunosorbent assay (ELISA) format as outlined in Figure 1 and graphically depicted in Figure 2.

Benefits

- Rapid, easy assay setup with an ELISA workflow
- No BSL-3 or cell culture required
- Results consistent with data from plaque reduction neutralization test

An HRP-conjugated fragment of the receptor-binding domain (RBD) of the spike protein is combined with a patient serum sample, which may contain neutralizing antibodies. This mixture is then added to the ELISA plate, which is coated with ACE2 receptor. If antibodies in the sample have neutralizing activity, binding of the HRP-RBD to ACE2 will be disrupted, and subsequent washing will remove the HRP-RBD. The resulting signal, measured using an absorbance microplate reader, will be lower in the presence of neutralizing antibodies, and comparison of the signal to that of assay controls enables assessment of the neutralizing activity present in the test samples.[3]

Here, results for the sVNT kit detected with SpectraMax® microplate readers and analyzed using SoftMax® Pro Software are presented. These results correlate closely with results previously generated using the traditional PVNT method, while only requiring a small fraction of the time and a less restrictive laboratory biosafety level.

Figure 1. *Comparison of PRNT and sVNT kit workflows. The sVNT assay can be completed in one hour in a BSL-2 lab, while PRNT requires live SARS-CoV-2 virus and cumbersome cell culture techniques, taking more than two days at a BSL-3 lab.*

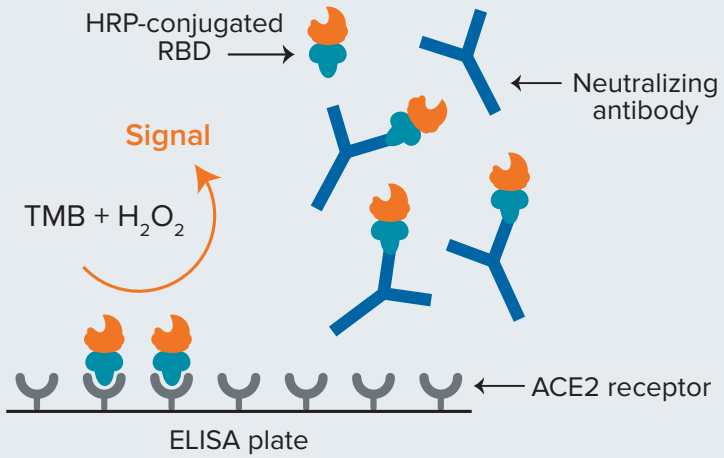

Figure 2. *SARS-CoV-2 sVNT method. The assay uses an ELISA format that enables detection of disruption of RBD binding to ACE2 by neutralizing antibodies in a test sample.*

Materials

- GenScript cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit (GenScript cat. #L00847-A)
- Serum samples (10) confirmed positive for neutralizing activity by PRNT (Corgenix)
- Serum samples (3) confirmed negative for neutralizing activity by PRNT (Corgenix)
- MultiWash+™ Microplate Washer (Molecular Devices)
- Molecular Devices microplate readers with absorbance detection mode:
  - SpectraMax® ABS Plus Microplate Reader
  - SpectraMax® iD5 Multi-Mode Microplate Reader
  - SpectraMax® i3x Multi-Mode Microplate Reader
  - SpectraMax® M5e Multi-Mode Microplate Reader

Methods

All kit components and test samples were allowed to reach room temperature prior to assay set up.

Kit reagents requiring dilution were prepared as follows:

- 1X wash solution was made by diluting the 20X stock in deionized water
- HRP-RBD stock solution was diluted 1:1000 in HRP Dilution Buffer to make the HRP-RBD working solution

Test samples and assay controls were mixed with HRP-conjugated RBD and incubated as follows. Test samples, along with positive and negative controls, were diluted 1:10 with sample dilution buffer, e.g., 12 µL test sample + 108 µL buffer. Each diluted sample and control was mixed in separate tubes with an equal volume of HRP-RBD working solution, e.g., 120 µL HRP-RBD working solution + 120 µL diluted test sample or control. Mixtures were incubated at 37°C for 15 minutes.

100 µL of positive control mixture, negative control mixture, and sample mixtures were added to duplicate wells of the assay strips. The plate was then sealed and incubated at 37°C for 15 minutes.

The MultiWash+ plate washer was used to wash all wells four times with 300 µL of 1X wash solution per well, followed by a final crosswise aspiration. 100 µL of TMB Solution was then added to each well, and the plate was sealed and incubated in the dark at room temperature for 15 minutes.

Cutoff	Result	Interpretation
≥ 30%	Positive	SARS-CoV-2 neutralizing antibody detected
< 30%	Negative	No detectable SARS-CoV-2 neutralizing antibody

Table 1. *Percent inhibition cutoff values used to interpret assay results.*

After incubation with TMB substrate, 50 µL of stop solution was added to each well, and absorbance at 450 nm was read immediately on each microplate reader.

Validity of the test results was assessed by determining whether the negative control OD_{450} values were above 1.0, and the positive control values were below 0.3. Failure to meet these criteria would invalidate the results of the test and require it to be repeated.

Percent inhibition for each test sample was calculated using the following formula:

$$Inhibition = (1 - \frac{OD\ value\ of\ Sample}{OD\ value\ of\ negative\ control}) \times 100\%$$

The cutoff values in Table 1 were used to interpret sample results as positive or negative, interpreted as the presence or absence of detectable SARS-CoV-2 neutralizing antibody. Cutoff values shown in the table are from the SARS-CoV-2 sVNT kit manual. For different geographic locations or ethnic backgrounds, users are directed to set up their own cutoff values based on representative patient serum panels.

Results

Quality of the assay results was assessed using the negative and positive controls. As shown in Table 2, the OD_{450} values for the negative control wells were above 1.0, and OD_{450} values for the positive control wells were below 0.3, as specified in the quality control requirements for the sVNT kit.

The OD_{450} values of the samples and of the negative control were used to calculate percent inhibition, as described in Methods. Percent inhibition values at or above 30% were interpreted as positive for the presence of SARS-CoV-2 neutralizing antibody, and values below 30% were interpreted as negative, based on the general cutoff values specified in the kit manual. Each of the three samples that had tested negative for neutralizing activity via PRNT also tested negative with the sVNT assay. Likewise, ten samples that tested positive via PRNT also tested positive via sVNT (Table 3).

Control	OD_{450}	Average	Std Dev	%CV
Negative	2.066 2.148	2.107	0.058	2.7
Positive	0.063 0.061	0.062	0.002	2.8

Table 2. *Negative and positive control OD_{450} values were above 1.0 and below 0.3, respectively, validating the assay results. Controls were run in duplicate, with %CV values below 3.0 demonstrating reproducibility.*

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	Plaque reduction neutralization test	GenScript sVNT assay		
Sample	PRNT90	Average OD_{450}	% Inhibition	Result
N-1	<20	1.991	5.5	Negative
N-2	<10	1.869	11.3	Negative
N-3	<20	1.590	24.6	Negative
P-1	160	0.112	94.7	Positive
P-2	80	0.061	97.1	Positive
P-3	80	0.096	95.4	Positive
P-4	160	0.091	95.7	Positive
P-5	320	0.084	96.0	Positive
P-6	320	0.141	93.3	Positive
P-7	160	0.105	95.0	Positive
P-8	640	0.101	95.2	Positive
P-9	640	0.149	92.9	Positive
P-10	160	0.225	89.3	Positive

Table 3. *Percent inhibition and positive vs. negative results for three samples that previously tested negative, and ten samples that tested positive, with a plaque reduction neutralization test (PRNT). Results shown were obtained using the SpectraMax ABS Plus reader; identical results (positive vs. negative) were obtained with the other SpectraMax readers.*

Conclusion

The GenScript cPass SARS-CoV-2 Neutralization Antibody Detection Kit data obtained using a set of patient serum samples matched results generated with the more traditional and laborious PRNT method. The sVNT kit offers easy-to-use reagents and a compact ELISA workflow that could be completed in about an hour. The one required wash step was automated using the MultiWash+ plate washer to save valuable time. SpectraMax readers with SoftMax Pro Software generate data that can be analyzed using an assay-specific protocol with formulas configured to apply the required calculations and output results automatically.

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