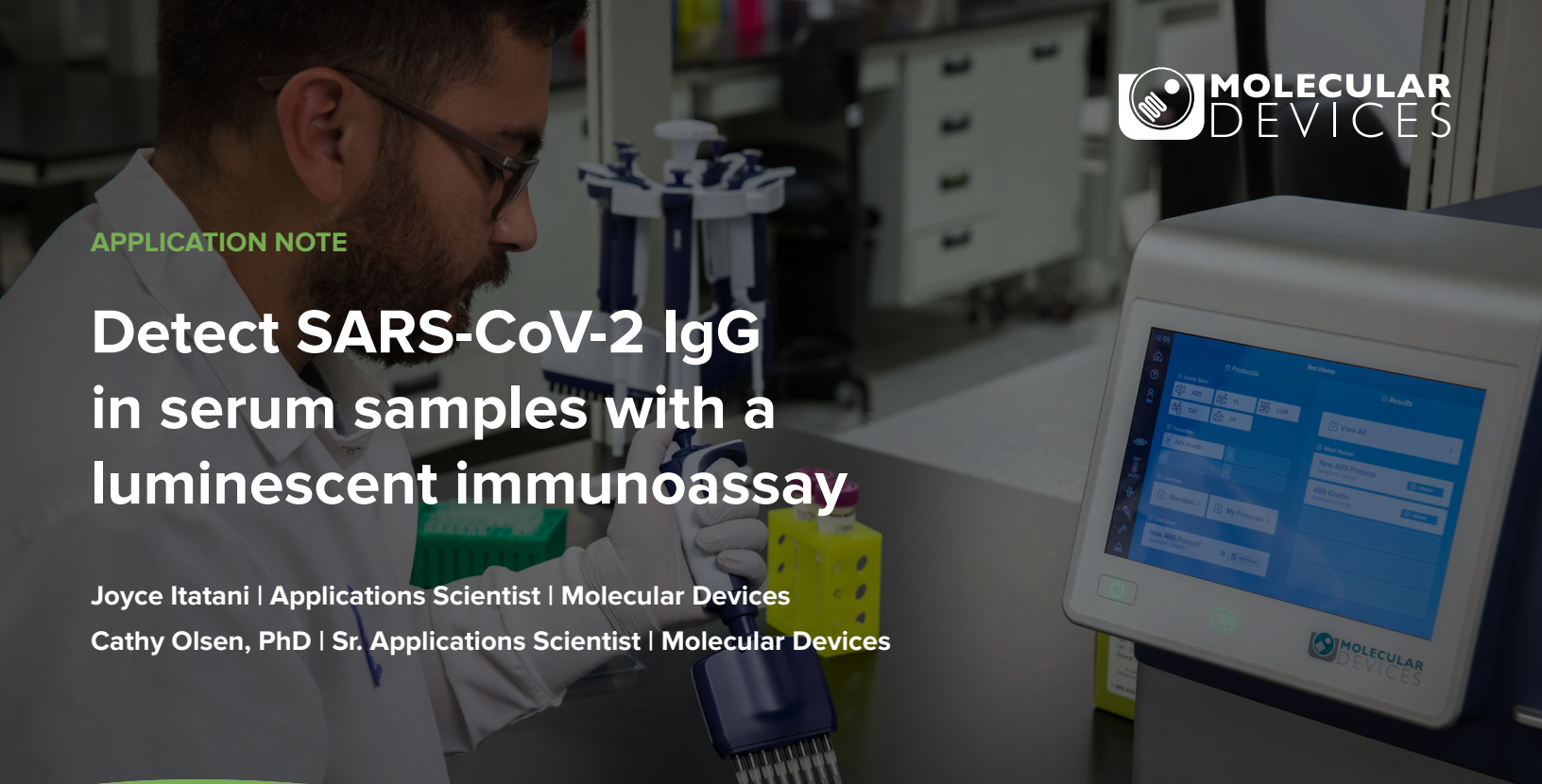

APPLICATION NOTE

Detect SARS-CoV-2 IgG in serum samples with a luminescent immunoassay

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Introduction

As part of the immune response to SARS-CoV-2, infected individuals produce virus-specific antibodies that are detectable in the blood within one to three weeks after the onset of symptoms. Both immunoglobulin-G (IgG) and IgM appear almost simultaneously, with IgG remaining at a high level for a longer time[1, 2]. The presence of detectable antibodies in serum resulting from infection or vaccination can protect against future infection, but the length of time these antibodies persist and the degree of protection they afford are among the many questions about SARS-CoV-2 that remain under intense investigation.

Immunoassays for the detection of SARS-CoV-2 antibodies in human serum are important tools for advancing our understanding of this disease, as well as vaccine research. The Promega Lumit™ Dx SARS-CoV-2 Immunoassay uses a fragment of the SARS-CoV-2 spike protein receptor binding domain (RBD) labeled with SmBiT and LgBiT, two subunits of NanoLuc® luciferase that is not functional until they are brought into close proximity via binding. When these labeled viral protein fragments are incubated with a sample containing SARS-CoV-2 antibodies, they bind to antibody, bringing the SmBiT and LgBiT subunits together to generate a functional luciferase. Adding the Lumit Detection Reagent generates a luminescent signal that is read on a microplate luminometer.

Benefits

- Scale up for HTS with a homogeneous, no-wash format
- Use a sample volume as little as 15 µL
- Obtain results in less than one hour

The simple, add-and-read format of the Lumit assay is depicted in Figure 1. There are no wash steps and results are generated in less than an hour, making the assay amenable to high-throughput screening. The SpectraMax® iD5 Multi-Mode Microplate Reader with ultra-cooled photomultiplier tube (PMT) offers highly sensitive luminescence detection of the Lumit Dx SARS-CoV-2 Immunoassay.

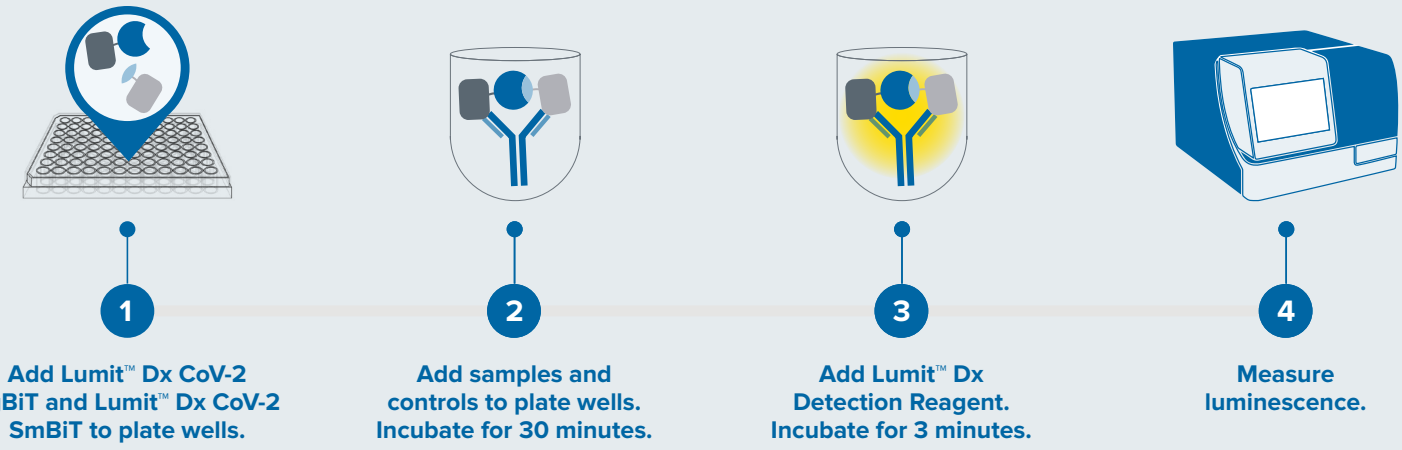

Figure 1. *Assay workflow for the Lumit Dx SARS-CoV-2 Immunoassay. The add-incubate-read format enables results in less than one hour and is amenable to automation and high-throughput screening.*

Materials

- Lumit Dx SARS-CoV-2 Immunoassay (Promega cat. #VB1080)
- COVID-19 positive serum samples (BioIVT cat. #HMSRM-COVID)
- Normal human serum (Amsbio cat. #HSER-2mL)
- 96-well white microplate (Greiner cat. #655075)
- SpectraMax iD5 Multi-Mode Microplate Reader (Molecular Devices)

Methods

Assay reagents were prepared and the plate was set up as follows (for additional details, please see the Lumit Dx SARS-CoV-2 Immunoassay technical manual):

1. 1X Lumit-Dx Immunoassay Dilution Buffer was prepared by adding 5 mL of 10X Lumit-Dx Immunoassay Dilution Buffer to 45 mL of Lumit™-Dx PBS/EGTA.
2. Serum samples from COVID-19 patients, or from a healthy donor, were diluted by combining 30 µL serum with 70 µL of 1X Lumit-Dx Immunoassay Dilution Buffer. Enough volume was prepared to run samples in triplicate; typically samples are run in single wells, requiring only 15 µL per sample.
3. Lumit-Dx CoV-2 BiTs Master Mix was prepared immediately prior to use by combining in a tube the following: 5 mL of 1X Lumit-Dx Immunoassay Dilution Buffer, 9.4 µL of Lumit-Dx CoV-2 LgBiT (red cap), and 9.4 µL of Lumit-Dx CoV-2 SmBiT (blue cap). The tube was inverted gently to mix.
4. Lumit-Dx Positive Control, Lumit-Dx Negative Control, and Lumit-Dx Assay Calibrator were thawed and brought to room temperature immediately prior to use. Tubes were centrifuged briefly to bring contents to the bottom.
5. To a 96-well white plate, the following were added:
   - 40 µL of Lumit CoV-2 BiTs Master Mix to all assay wells
   - 20 µL of diluted patient serum samples in triplicate
   - 20 µL of Lumit-Dx Positive Control in triplicate
   - 20 µL of Lumit-Dx Negative Control in triplicate
   - 20 µL of Lumit-Dx Assay Calibrator in triplicate
6. The plate was covered and incubated for 20 minutes at room temperature.
7. After incubation was complete, Lumit-Dx Detection Reagent was prepared by combining 7 mL of 1X Lumit-Dx Immunoassay Buffer with 230 µL of Lumit-Dx Detection Substrate (gray cap) immediately prior to use. The tube was inverted gently to mix.

 |

8. 60 µL of detection reagent was added to the wells, and the plate was incubated for three minutes at room temperature.
9. The plate was read on the SpectraMax iD5 reader using the settings shown in Table 1. After the microplate was read, the relative luminescence units (RLUs) were displayed in the plate section of SoftMax® Pro Software. Wells of the plate were assigned to groups in a template and the resulting group tables were used to calculate and display the results.

Assay quality control was assessed as indicated in the kit's technical manual. The Lumit-Dx Assay Calibrator, as well as Lumit-Dx Positive Control and Lumit-Dx Negative Control, were used to ensure proper assay performance. The Positive Control must yield an RLU higher, and the Negative Control must yield an RLU lower, than that of the Assay Calibrator. If these conditions are not met, then the plate results are invalid.

Results

Assay results were calculated by taking the ratio of the relative light unit (RLU) of each test sample (S) by the mean RLU of the Assay Calibrator (C). A ratio (S/C) ≥ 1 indicated a sample was positive for SARS-CoV-2 antibodies, while a ratio < 1 indicated a sample was negative for SARS-CoV-2 antibodies. The mean RLU for the Assay Calibrator was 548; mean RLUs for the six COVID-19 patient samples ranged from 1787 to 14,155, and the RLU for the healthy donor was 86.

As shown in Table 2, all ratios for samples from COVID-19 patients were greater than 1, indicating positive results, while the sample from the healthy donor had a ratio less than 1, indicating a negative result.

Note: The assay plate was also read on SpectraMax i3x, SpectraMax M5, and SpectraMax L readers, and similar results were obtained.

Parameter	Selected settings
Read mode	LUM
Read type	Endpoint
Wavelengths	All wavelengths
Plate type	96-well standard opaque
PMT and optics	Integration time: 1000 ms Read height: 4.7 mm
More settings	Show pre-read optimization

Table 1. *Instrument settings for Lumit-Dx assays on the SpectraMax iD5 reader. The All Wavelengths setting (no wavelength selection) was chosen to maximize the luminescence detected by the reader. Read height was optimized prior to reading using the Read Height Adjustment setting in SoftMax Pro Software.*

Sample	Mean ratio (S/C)	% CV	Result
COVID-19 patient 1	3.26	1.3	Positive
COVID-19 patient 2	7.30	2.2	Positive
COVID-19 patient 3	19.80	0.2	Positive
COVID-19 patient 4	14.98	4.3	Positive
COVID-19 patient 5	25.83	6.3	Positive
COVID-19 patient 6	14.77	4.7	Positive
Healthy donor	0.15	9.5	Negative
Positive control	7.74	3.2	Positive
Negative control	0.25	5.6	Negative

Table 2. *Results of Lumit Dx SARS-CoV-2 assay of patient samples on the SpectraMax iD5 reader. All samples and assay controls were run in triplicate, with % CVs shown. Results were calculated and interpreted using SoftMax Pro Software.*

Conclusion

Rapid testing of large numbers of serum samples for SARS-CoV-2 antibodies will be required as researchers endeavor to learn more about the timing and duration of the immune response against the virus by studying samples from the large number of individuals infected with or vaccinated against the virus. The Lumit Dx SARS-CoV-2 Immunoassay provides scientists a simple workflow for faster results. Used in conjunction with the SpectraMax iD5 reader and SoftMax Pro Software, calculations and interpretation of results are automated to enable higher throughput with less hands-on time.

References

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2. Long QX, Liu BZ, Deng HJ et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nat Med* 26, 845–848 (2020).

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