Comparative assessment of the binding and neutralization activity of bispecific antibodies against SARS-CoV-2 variants

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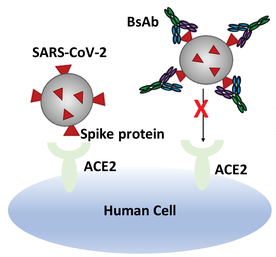
Introduction

Bispecific antibodies (BsAbs) against SARS-CoV-2 represent a promising class of therapeutics in the fight against COVID-19 due to their ability to target two non-overlapping epitopes on the spike protein.



BsAbs bind to two distinct sites at once on the spike protein to block ACE2 interaction.

The complexity associated with this class of antibodies requires adequate bioassays to ensure product quality and manufacturing consistency. While there are several available techniques for assessing potency of BsAbs, such as surface plasmon resonance (SPR) and the plaque reduction neutralization test (PRNT), these methods can be technically demanding, low throughout, and time-consuming, thus limiting their applications for lot release testing in quality control (QC) laboratories. This poster highlights our recent study in evaluating alternative techniques that could be used to assess the binding and neutralizing activities of BsAbs [1]. Here, we couple biolayer interferometry (BLI) and a focus reduction neutralization test (FRNT) to demonstrate orthogonal techniques that may be adoptable by stakeholders for quality assessment of BsAbs against SARS-CoV-2.

Understanding the Spike Protein and BsAbs

The spike (S) protein on the virus surface exists as a trimer and drives interactions with host cell receptors using the receptor binding domain (RBD) and internalization through confirmational changes of the S1 and S2 proteins. This makes it a critical target for COVID-19 therapeutics, including the BsAbs in our panel that each bind to either the receptor binding site (RBS) that recognizes the human receptor ACE2, the RBD, or the N-terminal domain of the S protein [2,3].

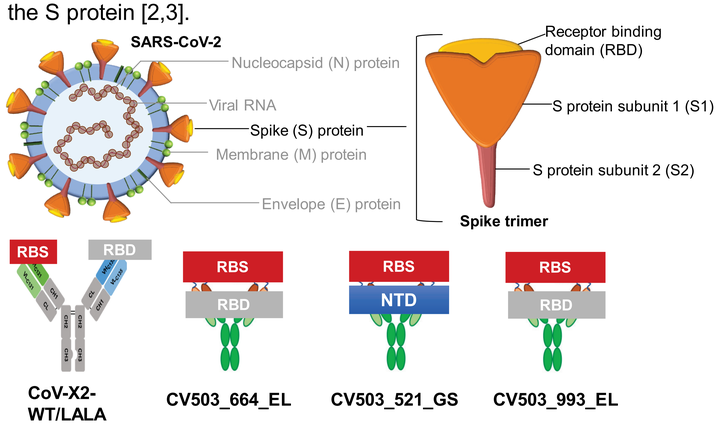


Figure 2. SARS-CoV-2 and the BsAbs. The structure of the S protein on the SARS-CoV-2 surface and respective binding epitopes of each arm of the BsAbs in the panel.

Biolayer Interferometry for S-Binding Interactions

We utilized biolayer interferometry (BLI) to characterize the binding affinity of each BsAb with Ancestral, Delta (B.1.617.2), and Omicron (B.1.1.529). BLI is an optical analytical technique that provides rapid, real-time kinetic analysis between two partners, including association, dissociation, and equilibrium dissociation constants.

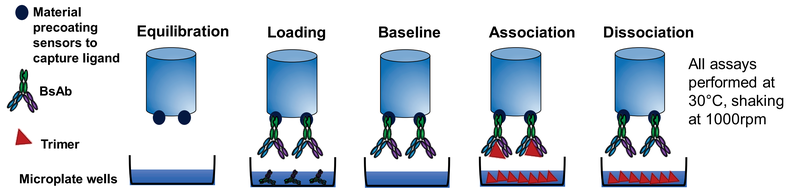
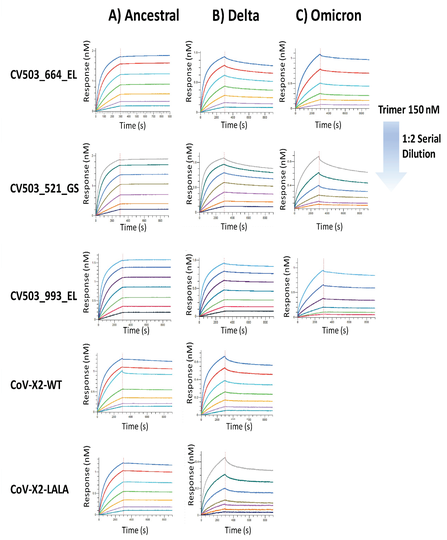


Figure 3. Summary of the BLI assay. Anti-human Fab2G biosensors were equilibrated in 1X kinetics buffer before capturing the CH1 domain of each BsAb. We then established a baseline, followed by association with serial dilutions of full-length S trimer, and dissociation in 1X kinetics buffer

BsAbs Display Variant-Specific Binding Affinity

CV503_664_EL	Ka	Koff	KD (nM)
Ancestral	$4.8x10^{4}$	$<1x10^{-7}$	$<1x10^{-12}$
Delta	$9.2x10^{4}$	$3.7x10^{-4}$	4.2
Omicron	$5.1x10^{4}$	$2.6x10^{-4}$	5.2
CV503_521_GS			
	Ka	Koff	KD (nM)
Ancestral	$5.3x10^{4}$	$<1x10^{-7}$	$<1x10^{-12}$
Delta	8.8x104	$6.9x10^{-4}$	8.0
Omicron	$6.5x10^{4}$	$9.3x10^{-4}$	14.5
CV503_993_EL			
	Ka	Koff	KD (nM)
Ancestral	$7.0x10^{4}$	$<1x10^{-7}$	$<1x10^{-12}$
Delta	$8.6x10^{4}$	$1.3x10^{-4}$	1.6
Omicron	$3.7x10^{4}$	$2.2x10^{-4}$	6.0
CoV-X2-WT			
	Ka	Koff	KD (nM)
Ancestral	$5.4x10^{4}$	$1.25x10^{-4}$	2.4
Delta	$9.4x10^{4}$	$3.3x10^{-4}$	3.5
CoV-X2-LALA			
	Ka	Koff	KD (nM)
Ancestral	$5.2x10^{4}$	$1.2x10^{-4}$	2.4
Delta	$9.1x10^{4}$	$4.6x10^{-4}$	5.1



- **All BsAbs bind to Ancesteral trimer with high affinity, ranging from subnanomolar to 2.4nM**
- **Binding is only slightly reduced against delta with the lowest affinity being CV503_521_GS with 8.0nM**
- **Binding is retained between Omicron and CV503 BsAbs, but abolished between CoV-X2 BsAbs and Omicron**

Figure 4. Binding Kinetics of BsAbs with SARS-CoV-2 variants. Kinetics experiments were performed using BLI with bispecific antibodies against SARS-CoV-2 trimer proteins. Representative sensorgrams shown of binding of BsAbs with **(A)** Ancestral, **(B)** Delta, and **(C)** Omicron variants. Due to lack of binding signals, CoV-X2 antibodies against Omicron is not displayed. Experiments were run in duplicate. The table provides the association (Ka), dissociation (Koff), and equilibrium dissociation constants (KD).

Disclaimer

This presentation reflects the views of the author and should not be construed to represent FDA's views or policies.

Focus Reduction Neutralization Test (FRNT)

H1299-hACE2 cells infected with known concentrations of SARS-CoV-2 isolates were seeded in a microplate and treated with BsAb dilutions, positive control, or left untreated.

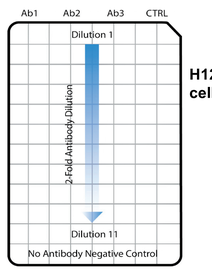
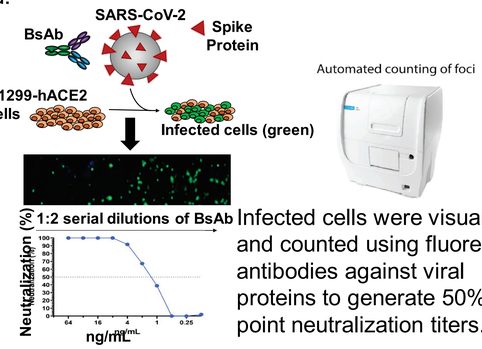
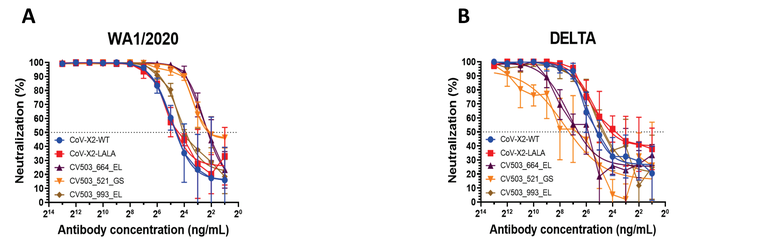


Figure 5. FRNT assay design



Infected cells were visualized and counted using fluorescent antibodies against viral proteins to generate 50% end-point neutralization titers.

BsAbs Neutralize Ancestral and Delta Variants



	CoV-X2-WT	CoV-X2-LALA	CV503_664_EL	CV503_521_GS	CV503_993_EL
FRNT50 (ng/mL)	25	34	6	7.5	28
IC50 (ng/mL)	30.01	34.12	5.667	10.12	20.12
R^2	0.8481	0.9116	0.9528	0.9482	0.9599

BsAbs potently neutralize Ancestral SARS-CoV-2 (WA1/2020) with FRNT50 and IC50 values both ranging from ~6-34ng/mL.

	CoV-X2-WT	CoV-X2-LALA	CV503_664_EL	CV503_521_GS	CV503_993_EL
FRNT50 (ng/mL)	64	27	144	192	34
IC50 (ng/mL)	54.8	45.45	160.5	203.6	34.65
R^2	0.9186	0.8718	0.8537	0.742	0.8904

BsAbs neutralize Delta (B.1.617.2) with FRNT50 and IC50 values ranging from 27-192ng/mL and ~35-204ng/mL, respectively.

Figure 6. Neutralizing Activity of BsAbs. FRNT assay performed for all BsAbs against **(A)** Ancestral and **(B)** Delta. Lack of neutralizing activity observed against Omicron (not shown).

Conclusions

We demonstrated that BLI and FRNT can be used in tandem to generate rapid and relevant data on the binding and neutralizing activities of BsAbs against SARS-CoV-2. We have observed similar trends between the two methods, where higher binding affinity typically results in higher potency against a variant, except in the case of Omicron. Our findings align with previously published data using SPR and PRNT assays, indicating that BLI and FRNT are orthogonal methods that can complement the commonly used assays for quality assessment of anti-SARS-CoV-2 products. With the growing field of BsAbs, which encompasses various production platforms, targets, and mechanisms of action, there is an increasing need for additional techniques for potency assays.

References

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2. De Gasparo, Raoul et al. Bispecific IgG neutralizes SARS-CoV-2 variants and prevents escape in mice. *Nature* vol. 593 (2021): 424–428.
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Acknowledgements

We would like to acknowledge Dr. Joshua Tan of NIH/NIAD and Dr. Luca Varani of the University of Switzerland for providing the CV503 and CoV-X2 BsAbs, respectively.