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WHITE PAPER

Addressing Viral Pandemics Such as COVID-19 Using the Carterra® LSA™

- **Understand mechanism of action (MOA) for therapeutic cocktails**
- **Characterize kinetics, epitope, blocking, and specificity on a single platform**
- **Leverage critical antibody-antigen insights to evaluate preclinical/clinical outcomes**
- **Fully characterize thousands of samples in a week using ultra-low sample quantities**

Emerging Diseases Require New Tools

Medicines to treat and prevent infectious diseases cover a broad range of modalities from dosed therapeutics to vaccines. Biotherapeutics require a detailed knowledge of binding sites, or epitopes, on their respective antigens. When biotherapeutics are formulated as combination therapies, understanding mechanism of action (MOA) is critical for identifying the appropriate cocktail. For vaccines, similar challenges include understanding therapeutically relevant sites to target on the pathogen, given the sophisticated evasion mechanisms common with many diseases. The number of samples in both biotherapeutic and vaccine development, whether recombinantly derived or from *in vivo* sources, can quickly grow into hundreds if not thousands. Additionally, the questions being asked are multi-factorial including not only how tightly but also structurally where a binding event occurs on a pathogen motif. While functional assays will always remain critical in assessing criteria such as neutralization, to develop biotherapeutics and vaccines into optimized and approved medicines requires detailed understanding of why these functional outcomes are observed.

COVID-19 has highlighted the severe time constraints a pandemic of this nature places on traditional means of therapeutic discovery. The rapid spread of this virus has required an approach to therapeutic and vaccine development measured in weeks rather than years. Further complicating efforts is the uniqueness of therapeutically relevant epitopes SARS-CoV-2 presents, which dictate that existing medicines may be limited in their effectiveness. Novel mutations in the SARS-CoV-2 spike protein have increased affinity towards the ACE-2 receptor and are attributed to increases in morbidity and mortality rates compared with SARS-CoV-1, making identification of viable medicines even more critical[1]. Therefore, combating rapidly emerging infectious diseases necessitates both increased throughput but also a detailed understanding of MOA at a much earlier stage of therapeutic development than has been done historically. This whitepaper highlights key considerations for how Carterra's technology, including the LSA™ label-free platform, is ideally suited for infectious disease research.

"Knowing how well these antibodies bind to the target is important but understanding their mechanism of action and how different antibodies can complement each other will determine what ends up in the clinic."

Dr. Erica Ollmann Saphire

Director, Coronavirus Immunotherapy Consortium (CoVIC), La Jolla Institute of Immunology

In the Trenches Against COVID-19

As COVID-19 has spread globally, researchers have quickly recognized the Carterra LSA's benefits of minimal sample requirements, speed, and incredible epitope resolution. Carterra has been selected by the Bill & Melinda Gates Foundation to provide full antibody characterization in their search for a COVID-19 therapeutic. The Coronavirus Immunotherapy Consortium (CoVIC) is a clearinghouse to analyze SARS-CoV-2 antibodies from collaborators worldwide towards identifying the most efficacious combinations. Carterra's LSA is the core technology the consortium is employing to rapidly characterize both affinity and epitope, supporting CoVIC's near term goal of developing a therapeutic cocktail as well as its long-term goal of identifying a vaccine based on highly potent antibodies. Duke University, a CoVIC partner, is also utilizing the LSA for

screening SARS-CoV-2 antibodies. CoVIC's strategy for antibody characterization on the LSA includes arraying antibodies on the sensor chip surface (up to 384 at once) and flowing the SARS-CoV-2 Spike protein across to assess binding kinetics. In a single experiment, up to 1152 antibodies can be screened for binding kinetics in this approach, which uses very little Spike antigen since a single injection tests for 384 on and off-rates in parallel. In epitope characterization studies done via competitive binning, antibodies are covalently attached to the sensor chip surface and in a pairwise fashion a mixture of the Spike protein and an antibody is passed across the array to test for sandwiching. These competitive binning assays test up to 384 antibodies in a single experiment, providing nearly 150,000 interactions used to understand epitope diversity and coverage. Heading the effort at CoVIC is Dr. Erica Ollmann Saphire who underscores why the LSA is essential to their mandate: "We are thrilled that Carterra will provide full antibody characterization data using affinity measurements and, potentially more importantly, ultra-high resolution information of the epitopes."

Incorporating the power of the LSA, but with a slightly different tactic, is South San Francisco's Distributed Bio. Distributed Bio has used their Tumbler™ technology to develop anti-COVID-19 antibodies based on known anti-SARS-CoV-1 sequences. This strategy, which included both modifying antibodies to target a novel virus as well as kinetic and epitope characterization studies using the LSA, was completed in only nine weeks and highlights how emerging disease challenges can be rapidly addressed with the appropriate screening tools. An example of SARS-CoV-2 Spike protein binding kinetics for a clone Distributed Bio identified that blocks ACE-2 interaction is seen in **Figure 1**[2].

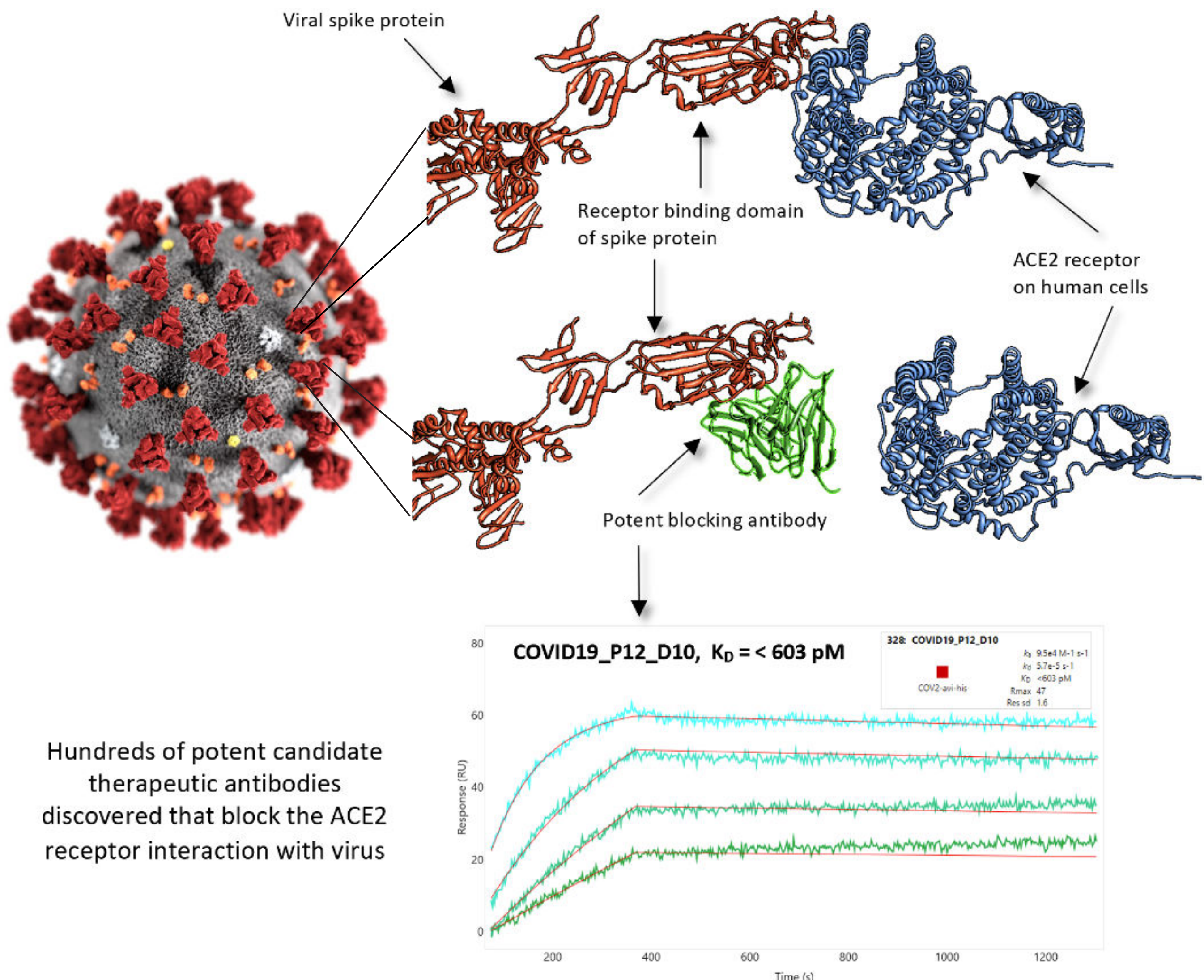

Figure 1: Data for sub-picomolar blocking clone found by Distributed Bio using the LSA[2].

At the outset of the COVID-19 pandemic, AbCellera (Vancouver, BC) and Eli Lilly (San Diego, CA) agreed to co-develop an antibody therapeutic against COVID-19. On June 1st, 2020, they announced Phase 1 dosing of a novel therapeutic antibody, LY-CoV555, discovered from this partnership. Significantly, LY-CoV555 is the first SARS-CoV-2 antibody trial in humans and development of this potential new medicine took only 3 months to reach Phase 1. Demonstrating a commitment to the speed needed in pandemics such as this, Eli Lilly has initiated large-scale manufacturing for LY-CoV555 while this Phase 1 is ongoing. Both AbCellera and Eli Lilly have incorporated the LSA as a key platform to identify binding affinity and epitope coverage from over 500 patient-derived antibodies, of which LY-CoV555 was derived from a COVID-19 survivor. Similar to the CoVIC initiative, these labs leveraged the throughput and minimal sample requirements of the LSA to speed up their research at both sites. In conjunction with neutralization assays, LSA data was used to better define MOA and focus on optimal antibody candidates.

A Primer on Carterra's Approach

The technology developed by Carterra performs and analyzes real-time binding analysis for up to thousands of biomolecules in a single experiment using surface plasmon resonance (SPR). While SPR has been commercially available for three decades, Carterra has developed a novel one-on-many fluidics configuration which inherently allows for high throughput sample characterization. In this design a sample can be screened for binding patterns against 384 surface-arrayed samples simultaneously. Additionally, injection of samples in sequence enable the formation and real-time monitoring of multi-constituent complexes which is particularly advantageous when studying binding site engagement by antibodies against antigens. Having hundreds of binding events per injected sample enables large scale studies of binding dynamics to be completed in days rather than months using traditional approaches. These high numbers of binding interactions monitored per sample injection also reduce the need to generate large quantities of antigen or antibody to support characterization efforts. Low sample requirements become critical for other sample types as well, such as serum, where less than 5 µl is required.

The LSA is Carterra's next generation HT-SPR™ system. This instrument can perform kinetic analysis for up 1152 antibodies and epitope characterization for up to 384 antibodies. The system operates in two fluidic modes to address the biosensor chip surface: multichannel mode to introduce 96 samples simultaneously to the chip surface and single channel mode where a single sample can be tested for binding against an array of up to 384 surface bound species. The LSA automatically alternates between multi and single channel mode depending on the assay design. Additionally, the system uses bidirectional flow, in which a fixed volume of sample is injected onto the chip surface in a back-and-forth flow pattern. This injection scheme minimizes sample consumption while enriching binding signals for even low-concentration samples. **Figure 2** shows why these key features result in the system being a highly efficient antibody characterization platform.

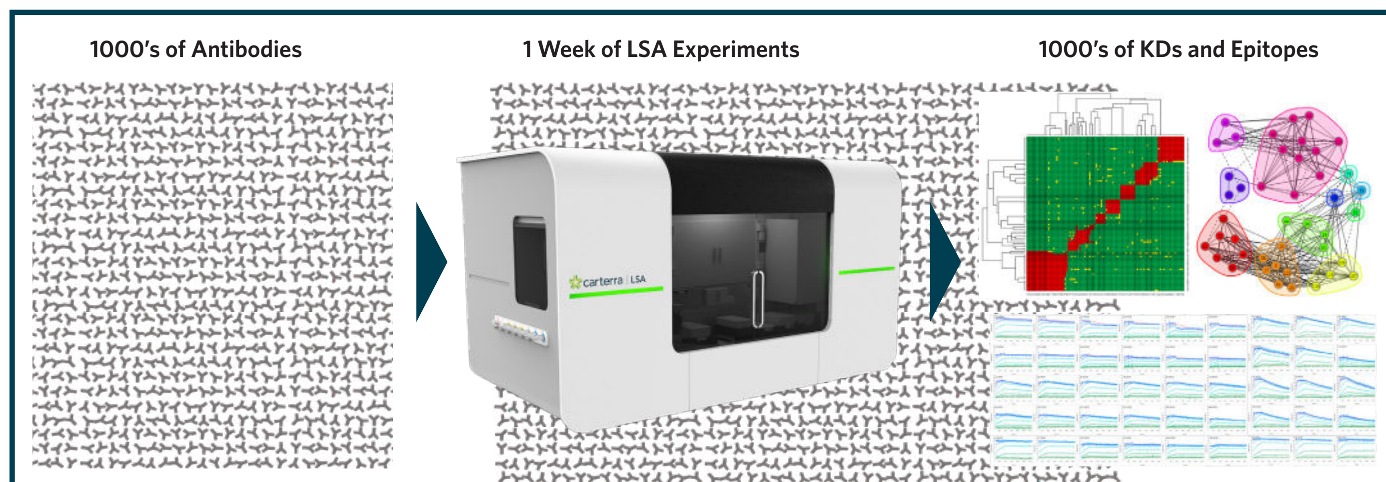

Figure 2: The advantages of comprehensive screening on the LSA to support infectious disease research.

Carterra's Technology in Infectious Disease

Based on a unique design which maximizes data per each injected sample, Carterra's one-on-many screening paradigm has made meaningful contributions to numerous studies focused on fundamental and applied vaccine and therapeutic strategies. **Table 1** briefly outlines peer-reviewed articles that have leveraged the Carterra's technology to answer complex and critical questions around infectious disease. These studies range from basic disease understanding, as in the study of therapeutically relevant *E. Coli* extracellular epitopes[11] to studies aimed at retrospectively evaluating unanticipated vaccine trial outcomes[10].

Manuscript	Impact of Carterra Technology
Wec et al. 2020[3]	Epitope binning to identify binding sites of longitudinal yellow fever vaccine B-cell response
Awasthi et al. 2019[4]	Mapping of HSV gD epitopes from serum of vaccinated mice
Cairns et al. 2017[6]	Epitope binning, mapping, and kinetics using mAbs to understand structural differences between HSV antigen forms
Cairns et al. 2019[7]	Discovery and epitope mapping of the HSV gD-gH/gL binding interface to enhance therapeutic and vaccine strategies
Chu et al. 2020[8]	Kinetic affinities of anti-HIV antibodies towards FcRs to aid in describing antibody effector function profiles elicited by IgG3 HL
Chukwuma et al. 2018[9]	Epitope binning of mAbs derived from HIV patients to identify broadly neutralizing cocktails
Hook et al. 2018[10]	Epitope monitoring of preclinical HSV vaccine immune responses
Storek et al. 2019[11]	Epitope binning/mapping and affinity characterization to understand therapeutic targeting of an extracellular *E. Coli* protein
Vij et al. 2018[12]	Affinity and epitope binning to characterize *E. Coli* mAbs targeting rare extracellular epitopes
Boesch et al. 2016[13]	Affinity of NHP vaccine model FcRs vs. human FcRs
Yeung et al. 2016[14]	Epitope binning of IgGs towards understanding predisposed innate immunity against an *S. aureus* antigen

Table 1: Summary of peer-reviewed research applying Carterra's technology to infectious disease.

Characterization of Therapeutic Antibodies

The one-on-many technology readily lends itself to studies characterizing the affinity and epitope of antibodies towards their respective antigen(s), as noted for several examples in **Table 1**. In these studies, purified antibodies from a variety of sources and platforms are arrayed on the biosensor chip surface either covalently through amine coupling chemistries or non-covalently typically by capture using the Fc region of the antibody, as shown in **Figure 3**. For most experiments, 2 µg or less of each antibody is required to create the array. Given the high capacity for unique samples on the sensor chip surface, additional positive and negative controls are also typically included to better understand assay properties and further support conclusions. One additional benefit is that these assay formats present the antigen in the solution phase against the antibody array which is ideal for unhindered presentation of antigen epitopes.

Affinity Measurements

In studies focused on affinity characterization, either by binding kinetics or steady state analysis, an ascending titration of antigen is injected across the array. For examples see Storek et al[11] and Vij et al[12] in **Table 1.** Determining binding affinities for up to 384 antibodies typically requires less than 20 µg total of antigen. Assay run times to determine 384 unique affinities using this multiple titrant approach average approximately 12 hours or less. If the antibody panel in question contains less than 384 clones, the LSA's capacity then affords an opportunity to include replicates thereby increasing statistical confidence in measured affinities. Carterra's Kinetics™ software is designed specifically for detailed processing and fitting of hundreds of affinity interactions in a few simple steps. Assay processing typically takes less than an hour, even for panels containing hundreds

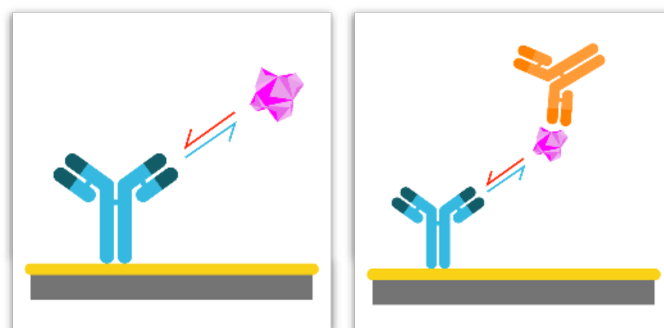

Figure 3: Immobilization of antibodies on the sensor chip surface allows high numbers of samples to be arrayed while presenting the antigen as an injection to optimize accuracy of binding data. Additional injections can be layered on top of the antigen including receptors or competing mAbs.

of antibodies. With thoughtful assay design, measured affinities demonstrate excellent correlation with solution-based approaches, minimizing the need for follow up assays using traditional lower throughput techniques[5]. The high accuracy of affinities measured on the LSA enables consideration of therapeutically relevant affinity ranges and dosing strategies at the earliest stages of therapeutic discovery.

Epitope Binning and Mapping

As a complement to understanding how tightly antibodies engage a therapeutically relevant site on a pathogen, epitope characterization is a central technique for classifying antibodies based on similarities in antigen binding sites. In competitive epitope binning studies, the ability of two antibodies to bind the antigen simultaneously is assessed in a pairwise fashion for all constituents of a panel. The assay tests each antibody both on the surface as well as in solution to increase confidence in the grouping assignments, termed epitope bins. Panels of up to 384 constituents can be competed against themselves, achieving up to 150,000 real-time interaction profiles. The total antibody requirement for competitive binning assays is usually around 5 µg. Additionally, benchmark antibodies or native pathway binding partners to the antigen can be included in the assay to better localize binding sites and evaluate blocking capabilities of the antibodies. Carterra's Epitope™ software distills thousands of competitive outcomes into straightforward network plots displaying the intricate relationships among all candidates. Additionally, the software can incorporate data from external sources, allowing antibody attributes such as neutralization to be displayed in the context of epitope groupings. See Wec et al.[3] and Storek et al.[11] for excellent examples of competitive epitope binning assays and data outputs.

In order to localize antibody binding sites on an antigen domain or residue, mutant or epitope mapping utilizes antigen variants, subdomains, or peptides derived from an antigen. In mutant mapping experiments, altered antigen forms are injected across an antibody array to determine changes in binding versus an intact or wild-type form of the antigen. Depending on the type of mutation, these studies can map binding sites to either general regions or domains on the antigen structure or even to specific residues by using, for example, alanine scanning mutants. In epitope mapping studies **(Figure 4)**, rather than arraying antibodies, an overlapping peptide library derived from the antigen is immobilized. In each cycle of the experiment, an antibody is injected across the array. Depending on the size of the peptide overlaps, epitope mapping can give a resolution down to the single residue level. The Epitope™ software has dedicated modules for analyzing mutant and epitope mapping data sets.

Antigen Structural Characterization

While epitope characterization is most commonly employed to guide therapeutic candidate selection, the antibodies themselves can be used as tools to understand the pathogen. Cairns et al.[6,7] established antigenic sensing relationships for two forms of herpes simplex virus (HSV) glycoprotein D. By monitoring how certain antibodies changed in their epitope grouping assignments, this study was able to establish key structural insights for how these highly conserved proteins were distinct in their antigenic landscapes. Given the propensity for pathogens such as viruses to mutate and evade host immunity, this technique is a rapid means of understanding the structural implications of these changes without requiring slower and more intensive techniques such as X-ray crystallography.

Effector Function

Structurally opposite antigen binding domains of antibodies are the Fc regions which interact with Fc gamma receptors (FcRs) and play an important role in effector function. Characterizing FcR binding using Carterra's technology is commonly done by examining kinetic or steady affinities of FcR interactions with antibody Fc domains. In these studies, antibodies are captured via anti-Fab surfaces or else by direct amine coupling and then FcRs are injected across the array as a titration. These studies have provided seminal data in understanding differences in effector function of non-human primate models compared with humans and have highlighted mechanisms of immune response outside of antigen targeting alone[13]. In a single experiment using this format, multiple FcRs can be screened against a large array of antibodies, making efficient use of both the biosensor chip but also the FcRs, which can be costly to produce or procure. To further validate results, the Kinetics™ analysis software has features facilitating direct comparison of FcR affinities derived from both global kinetics and steady state fitting. This is particularly valuable when studying the rapid kinetics associated with FcR-antibody binding.

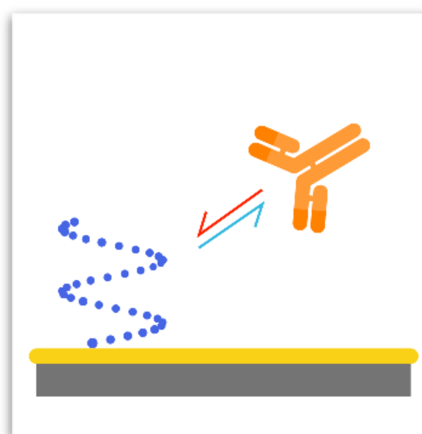

Figure 4: Epitope mapping takes advantage of high array capacity to immobilize up to 384 unique peptides on the sensor chip surface.

Characterization of Preclinical/Clinical Samples:

The antibody composition of biofluids are also amenable to analysis using Carterra's technology. Hook et al.[10] and Awasthi et al.[4] developed a process which first utilized epitope binning experiments to select a group of antibodies having diverse and carefully detailed antigen epitopes. This reference panel of antibodies was then arrayed on the biosensor surface and injections containing a mixture of the antigen and serum antibodies were passed over the array. The ability of the antigen to bind the surface antibodies indicated what epitopes

were being targeted by antibodies due to vaccination or infection. In these studies, the amount of serum required was approximately 5 µl or less, and therefore highly compatible with the limited volumes available from preclinical models. This approach allows longitudinal analysis of immune response epitopes under a variety of circumstances such as varied vaccination regimens to monitoring of changes over the course of disease infection.

Summary

The Carterra LSA is a real-time label-free biosensor unique from other technologies. The system is purpose built to characterize up to 384 samples with exquisite resolution. The capabilities of the LSA are well suited for long term infectious disease research objectives but with a throughput capability that allows the system to easily meet the limited timelines during rapidly evolving threats such as COVID-19. Additionally, the LSA's patented microfluidics enable it to characterize epitope and affinity using sample quantities far below other technologies. Speed in conjunction with high resolution is vital towards making informed decisions on hundreds if not thousands of samples during viral pandemics.

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For more information on the LSA and how it can be leveraged to enhance infectious disease studies, please contact Carterra at questions@carterra-bio.com.

Carterra technology is protected by the following patents and other patents pending:
8,210,119, 8,211,382, 8,383,059, 8,999,726, 9,682,372, 9,682,396

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