MaxCyte®: A Versatile Tool for SARS-CoV-2 Response

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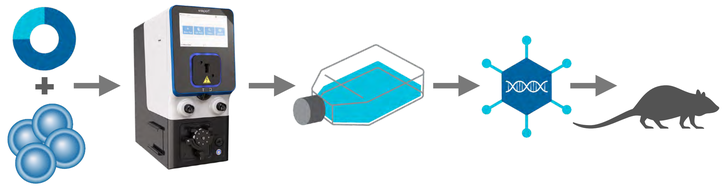
Abstract

The COVID-19 caused by SARS-CoV-2 has proven to be an incredible burden on global health care systems. New viral variants continue to emerge and spread worldwide, emphasizing the need for rapid, meaningful research to guide an effective pandemic response. Simultaneous development of fast, quantitative diagnostics to trace disease spread and prevalence combined with the generation of new vaccines promote a comprehensive approach to combating the virus. MaxCyte® electroporation is a versatile technology for both highly efficient protein production and cellular engineering. Here we present data illustrating how MaxCyte enabled not only novel vaccine development but also new diagnostic techniques and cell-based high throughput drug candidate screening. MaxCyte is an essential biotechnology platform empowering fundamental SARS-CoV-2 research.

Results

MaxCyte enabled vaccine development



Experimental Design

The AdCOVID vaccine candidate developed here is a replication-deficient adenovirus type 5 vector that expresses the human gene for the receptor-binding domain (RBD) for the SARS-CoV-2 spike[1]. Recombinant plasmids encoding viral genes were electroporated into PER.C6 cells using the MaxCyte STx™ instrument. Newly synthesized virus was purified from cell lysates. The safety and efficacy of the AdCOVID vaccine were determined through single intranasal dose administered in mice.

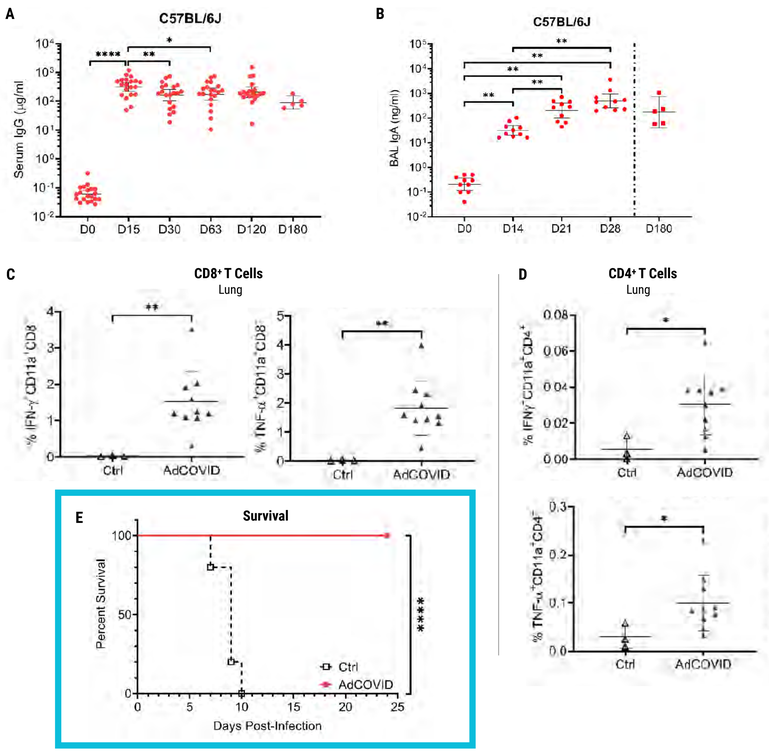
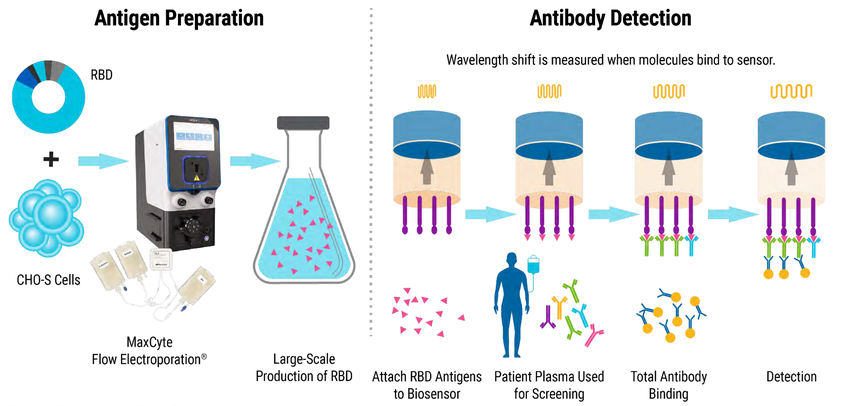


Figure 1: AdCOVID vaccine stimulates immune response and protects against lethal SARS-CoV-2 challenge. Single intranasal administration of the AdCOVID vaccine resulted in the generation of spike-specific A) IgG antibodies in sera and B) IgA antibodies in bronchoalveolar lavage (BAL) fluids in the circulation of C57BL/6J mice for at least six months. The AdCOVID vaccine also induced SARS-CoV-2 specific T cell response in outbred CD-1 mice. Both C) CD8+ and D) CD4+ T cells were present in the lung (and spleen data not shown) of vaccinated mice. T cells produced both interferon-γ (INF-γ) and tumor necrosis factor-α (TNF-α) upon restimulation with RDB peptide pool. E) AdCOVID vaccine protected K18-hACE2 mice from lethal SARS-CoV-2 infection. Mice were challenged intranasally 28 days post vaccination with the 2019-nCoV/USA-AZ1/2020 strain. All vaccinated mice survived 24 days post infection (DPI), while vehicle control mice succumbed to COVID19-like disease 10 DPI. Vaccination elicited antigen-specific antibody response and polyfunctional T cell activation, both systemically and locally, allowing complete protection against lethal SARS-CoV-2.

Rapid and sensitive detection of SARS-CoV-2 antibodies by biolayer interferometry



Experimental Design

Biolayer interferometry immunosorbent assay (BLI-ISA) is a new diagnostic for the detection of SARS-CoV-2 antibodies[2]. This new assay uses a simple automated process that takes less than 20 minutes and delivers real-time measurements of total antibody levels and specific antibody isotypes. CHO-S cells were transfected with an expression plasmid for RBD using MaxCyte Flow Electroporation®. The antigen was then harvested and purified from the cells. Biosensors were dipped into RBD antigens and used to test plasma for antibodies against SARS-CoV-2. As antibodies bind to antigens, real-time measurements within the sensor record the change in the reflected wavelength of light over time, resulting in a semi-quantitative measurement of the antibodies present in patient plasma.

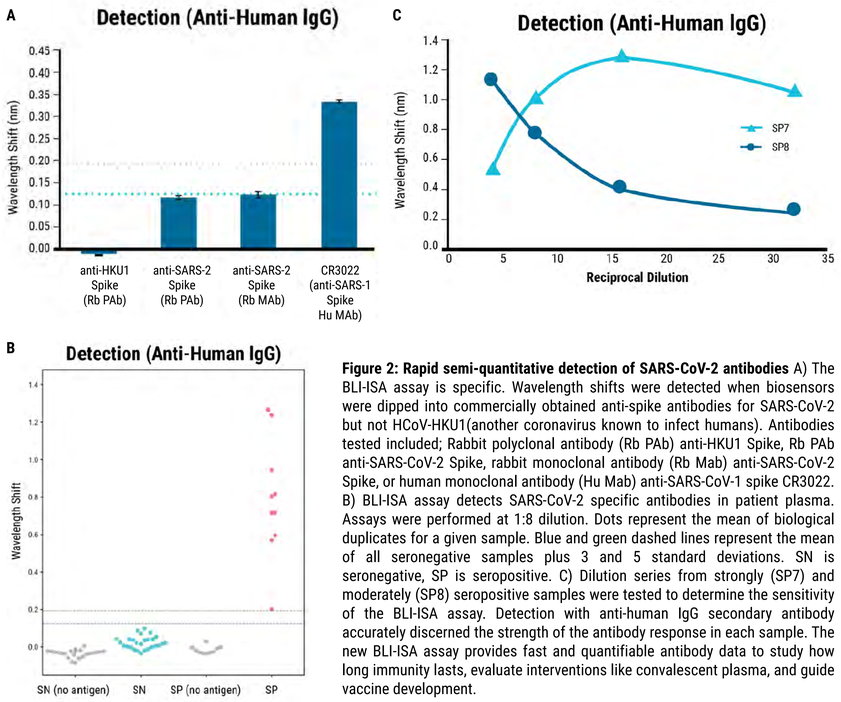
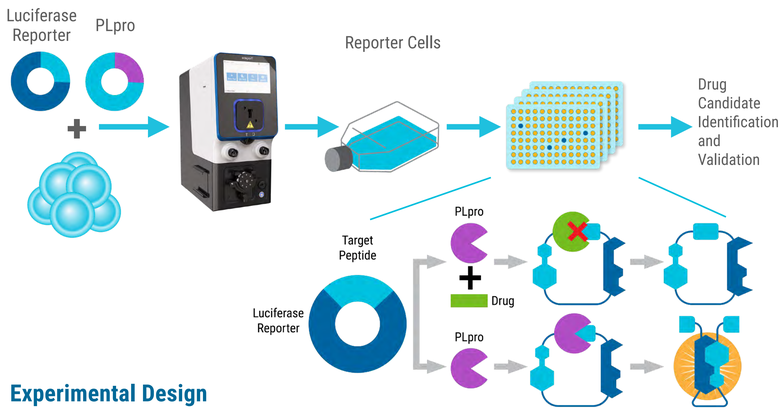


Figure 2: Rapid semi-quantitative detection of SARS-CoV-2 antibodies A) The BLI-ISA assay is specific. Wavelength shifts were detected when biosensors were dipped into commercially obtained anti-spike antibodies for SARS-CoV-2 but not HCoV-HKU1(another coronavirus known to infect humans). Antibodies tested included; Rabbit polyclonal antibody (Rb PAb) anti-HKU1 Spike, Rb PAb anti-SARS-CoV-2 Spike, rabbit monoclonal antibody (Rb Mab) anti-SARS-CoV-2 Spike, or human monoclonal antibody (Hu Mab) anti-SARS-CoV-1 spike CR3022. B) BLI-ISA assay detects SARS-CoV-2 specific antibodies in patient plasma. Assays were performed at 1:8 dilution. Dots represent the mean of biological duplicates for a given sample. Blue and green dashed lines represent the mean of all seronegative samples plus 3 and 5 standard deviations. SN is seronegative, SP is seropositive. C) Dilution series from strongly (SP7) and moderately (SP8) seropositive samples were tested to determine the sensitivity of the BLI-ISA assay. Detection with anti-human IgG secondary antibody accurately discerned the strength of the antibody response in each sample. The new BLI-ISA assay provides fast and quantifiable antibody data to study how long immunity lasts, evaluate interventions like convalescent plasma, and guide vaccine development.

A cell-based high throughput screening assay for SARS-CoV-2 drug candidates



Experimental Design

MaxCyte® electroporation (EP) enabled the development of a genetically engineered reporter cell line essential for a new cell-based high throughput screening platform. Electroporation allowed for the simultaneous delivery of an engineered firefly luciferase (Fluc) reporter plasmid and a plasmid expressing the SARS-CoV-2 papain-like protease (PLpro) into HEK293 cells. The Fluc reporter plasmid was engineered to express a target peptide sequence containing a PLpro cleavage site. Inhibitors of PLpro prevent the enzyme from cleaving its target sequence, hindering Fluc dimerization decreasing luminescence compared to untreated cells. Identified targets were further validated by additional enzymatic and cell-based assays[3].

HTS Campaign Statistics

Library	Stage	Concentration	# Samples	# Replicates	# Plates	Z'	S:B	Hit Cutoff	# Hits
ReFRAME	Primary	10 μM	13,104	1	11	0.71 ± 0.04	11.38 ± 1.57	35.1%	212
	Confirmation	10 μM	235	3	1	0.75	15.48	27.5%	210
	Titration	20 μM	210	3	6	0.72 ± 0.03	18.39 ± 1.30	IC_{50} < 10 μM	164
	Titration CS	20 μM	210	3	6	0.76 ± 0.02	3.02 ± 0.15	IC_{50} < 10 μM	185

CS:counterscreen, S:B: signal-to-background ratio

Table 1: Cell-based High Throughput Screening Statistical Analysis. A single transfection by MaxCyte® EP generated enough reporter cells to screen over 15,000 compounds from three separate libraries (ReFRAME, Pathogen Box and TargetMol®). Critically, this assay includes a cytotoxicity titration counterscreen to determine each drug candidate's effect on cell viability. This allows for the rapid detection of potentially hazardous drug candidates. Of the 13,104 candidates from the ReFRAME library, four potential compounds were found to partially inhibit the PLpro enzyme without significant risk to cell viability.

Summary

- MaxCyte® enabled the modeling of an effective prophylactic AdCOVID vaccine for SARS-CoV-2
- MaxCyte Flow electroporation® produced high quality antigens for a new rapid semi-quantitative diagnostic assay that can aid the study of SARS-CoV-2 prevalence
- MaxCyte scalability enabled the development of a cell-based high throughput screening platform to identify potential drug candidates for COVID-19 treatment
- MaxCyte electroporation is a versatile technology empowering fundamental SARS-CoV-2 research

References

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2. Dzimianski JV, Lorig-Roach N, O'Rourke SM, Alexander DL, Kimmey JM, DuBois RM. Rapid and sensitive detection of SARS-CoV-2 antibodies by biolayer interferometry. Sci Rep. 2020;10(1):21738. Published 2020 Dec 10. doi:10.1038/s41598-020-78895-x
3. Smith E, Davis-Gardner ME, Garcia-Ordonez RD, et al. High-Throughput Screening for Drugs That Inhibit Papain-Like Protease in SARS-CoV-2. SLAS Discov. 2020;25(10):1152-1161. doi:10.1177/2472555220963667

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MC-PO-WVC Rev 0 4/22