COVID-19 Antibodies Detection Using Spike Protein Microarray Chip

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Objective

Create a microarray chip to detect COVID-19 antibodies and determine the optimal antigen printing concentration for microarray-chip detection.

Background

- The COVID-19 pandemic has highlighted the significance of efficient testing and the development of high throughput technologies for the detection of antigens and antibodies that will allow diseases within the community to be accurately reported.
- Detailed antibody profiles can help in the creation of vaccines for the prevention of such diseases and can be useful for future studies involving body fluids.

Methods

- The sciFLEXARRAYER S3 antigen printing system was used to create 5x5 antigen printing block on the microarray chip. Each row contained a different concentration of COVID-19 spike protein. (Figure 1)

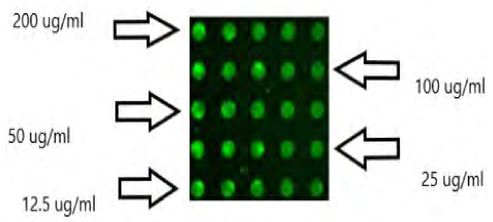


Figure 1 Schematic of methods used for experimentation

- In each spot, a primary RBD (receptor binding domain) antibodies bound to the antigen and the secondary Cy5-conjugated anti-mouse antibodies bound to the primary antibodies.

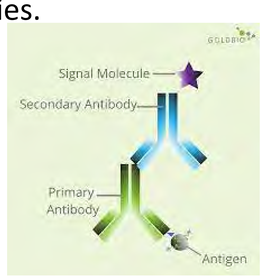


Figure 2 Schematic of Antigen-Antibody Complex

- There were 16 total blocks, arranged with 2 blocks containing the same concentration of antibodies, to test for reproducibility.
- The different concentrations of primary antibody were: 8 µg/ml, 4 µg/ml, 2 µg/ml, 1µg/ml, 0.5µg/ml, and 0.25 µg/ml (Figure 3).
- CD106 and PF4 mouse antibodies mixed in 1% BSA in PBS was used as negative control, while the blank well only contained 1% BSA wash.
- Software Genepix Pro was used for fluorescence detection and microarray analysis.

Results

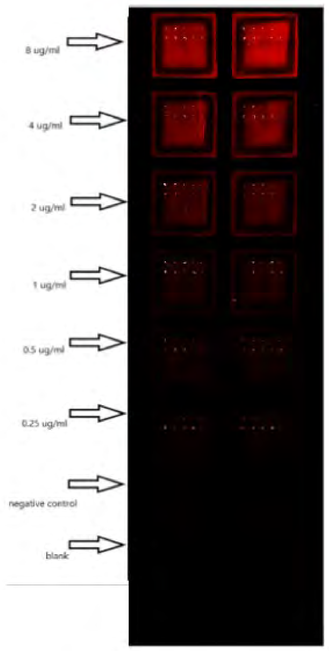
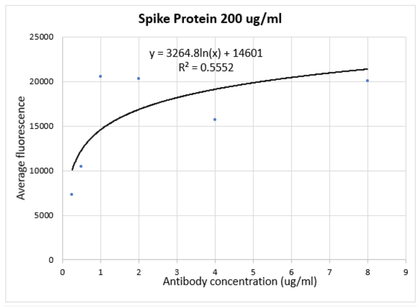
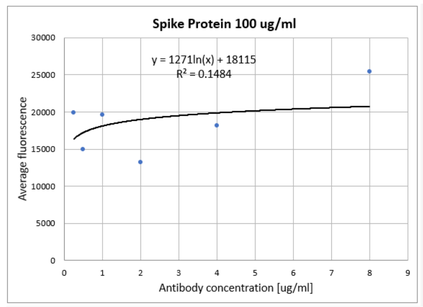


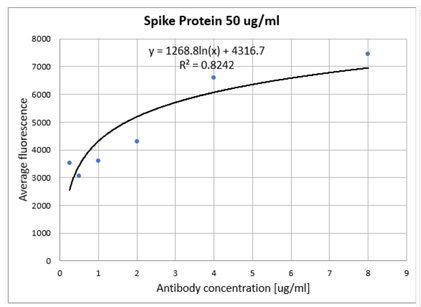
Figure 3. Fluorescence detection in the sixteen blocks.

The fluorescence decreased as the as the antibodies concentration decreased. Negligible fluorescence is detected in the negative control and blank wells confirming the success of the experiment and the proportional relationship between the antigen to antibody concentration.

STANDARD CURVES AND R-SQUARED VALUES







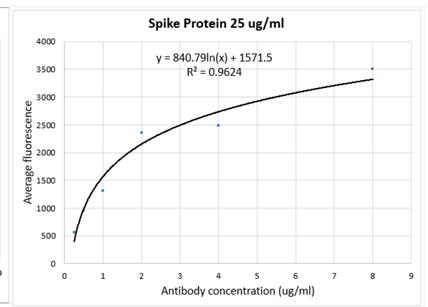
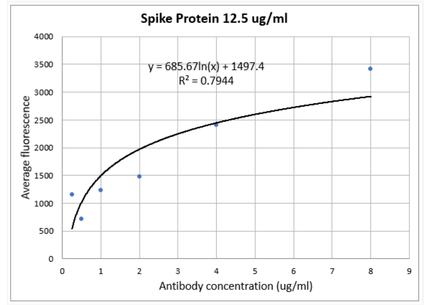


Figure 4. Using the average fluorescence and the antibody concentration, a standard curve was generated for each antigen concentration. The highest R-squared value was 0.96 and it was obtained from the standard curve of the 25 µg/ml of spike-protein, indicating this as the optimal antigen concentration for antibody detection.



LIMITS OF MICROARRAY CHIP DETECTION BASED ON OPTIMAL PRINTING CONCENTRATION

Using the equation of the standard curve for the spike protein concentration of 25ug/ml:
Lowest antibody concentration (X-axis) calculated: 0.30ug/ml
Highest antibody concentration detected: 10.0ug/ml
** Values were calculated using highest and lowest fluorescence detected (y-axis).

Conclusion

This project successfully detects the COVID-19 antibodies using the microarray chip. This is evident in the increased fluorescence activity observed with increasing antibody concentration within the spots of the microarray chip (Figure 3). 25ug/mL was determined as the optimal antigen concentration for antibody detection using this system (highest R-squared value).