

## Supplementary Information for

### A high-throughput multiplexed microfluidic device for COVID-19 serology assays

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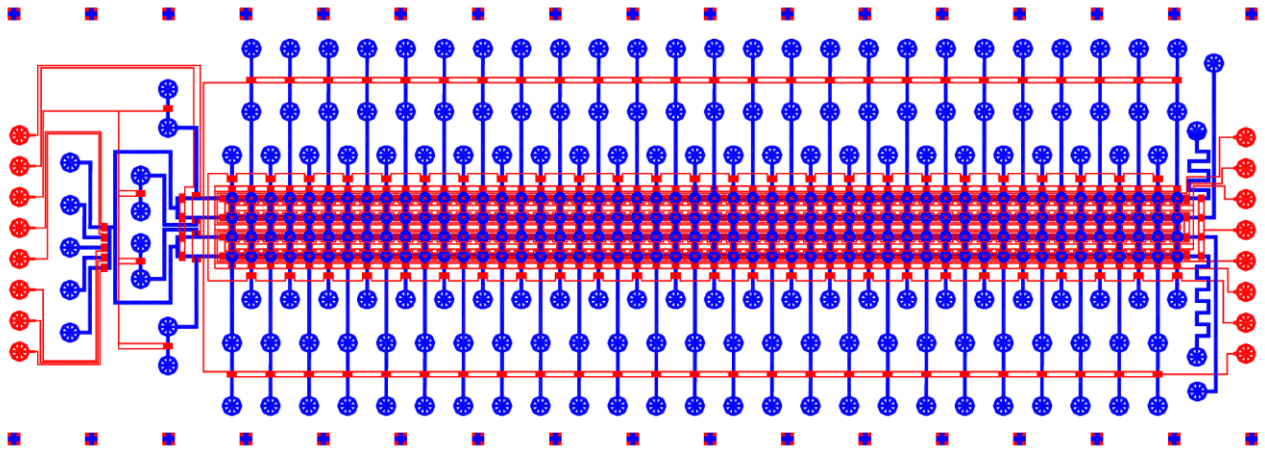
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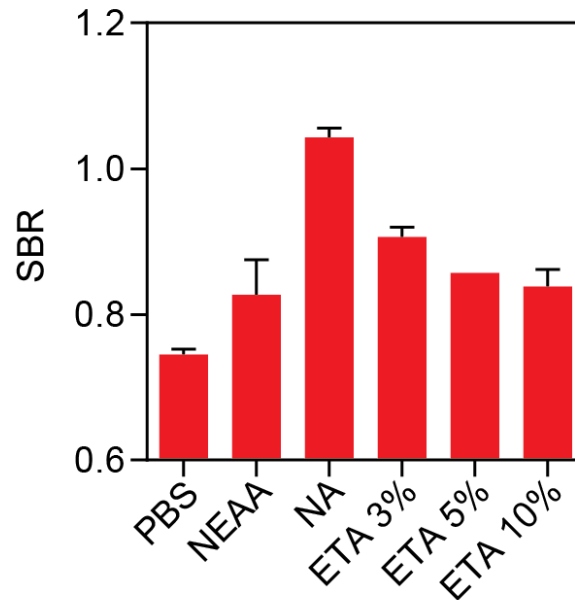
**Table S1. Patient Characteristics**

|                         | <b>Archival (n=34)</b> | <b>COVID-19 (n=66)</b> |
|-------------------------|------------------------|------------------------|
| Age mean (range)        | N/A                    | 55 (19 -80)            |
| Gender                  | N/A                    |                        |
| Male                    | N/A                    | 68% 45/66              |
| Female                  | N/A                    | 32% 21/66              |
| Mechanical ventilator   | N/A                    | 57% 24/42              |
| Sample collection date  | 2018                   | Apr-May 2020           |
| Symptoms                |                        |                        |
| Sudden symptom onset    | N/A                    | 49% 21/43              |
| Fever                   | N/A                    | 79% 34/43              |
| Dyspnea                 | N/A                    | 91% 39/43              |
| Diarrhea                | N/A                    | 21% 9/43               |
| Chest pain              | N/A                    | 28% 12/43              |
| Days post symptom onset | N/A                    | 13 days (4-54)         |
| Comorbidities           |                        |                        |
| Diabetes                | N/A                    | 40% 17/43              |
| Asthma                  | N/A                    | 0% 0/43                |
| Immunocompromised       | N/A                    | 7% 3/43                |
| Hypertension            | N/A                    | 13% 13/43              |
| Cardiovascular disease  | N/A                    | 9% 9/43                |
| Obesity                 | N/A                    | 23% 10/43              |
| Renal insufficiency     | N/A                    | 9% 4/43                |
| Smoker                  | N/A                    | 16% 7/43               |

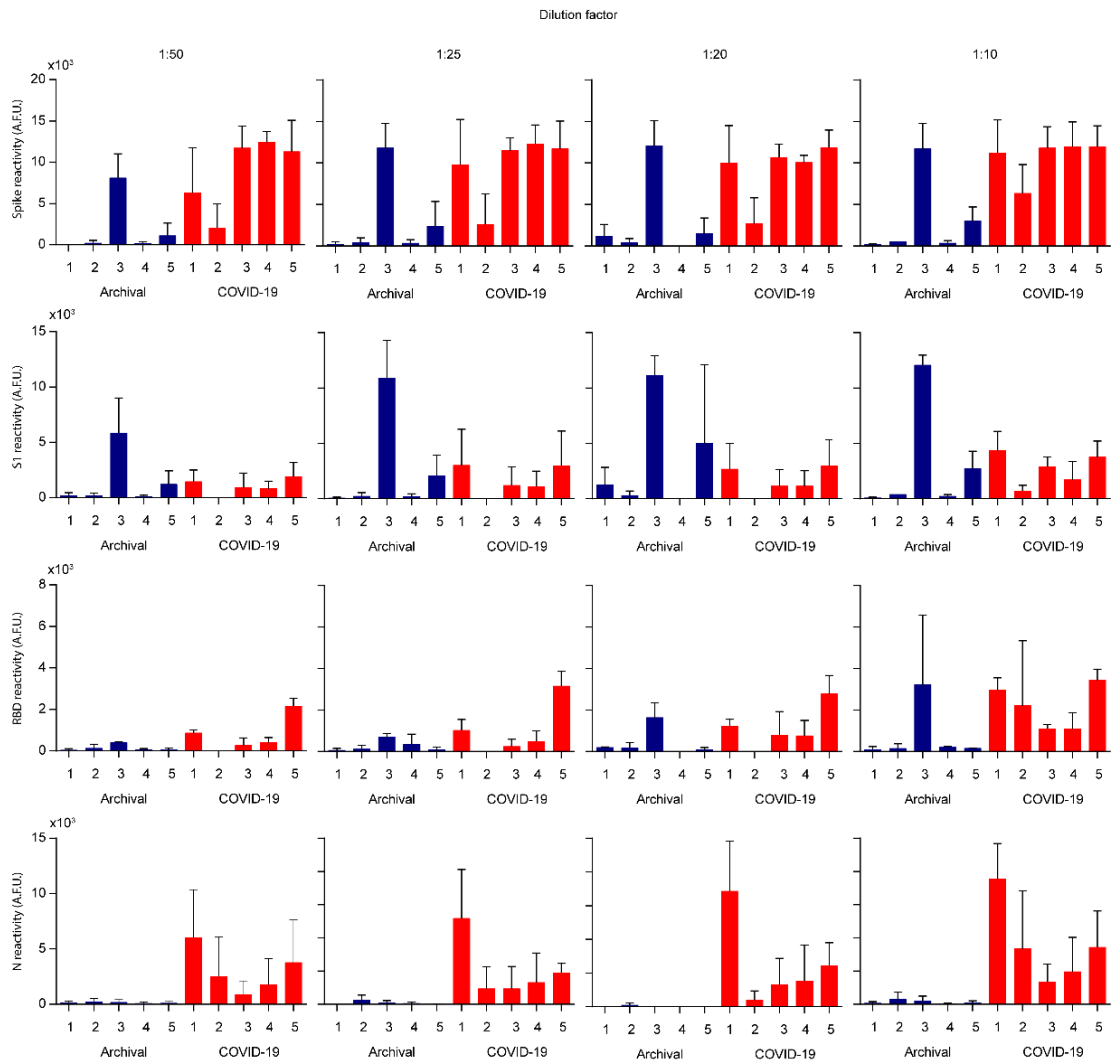
N/A, Not available.



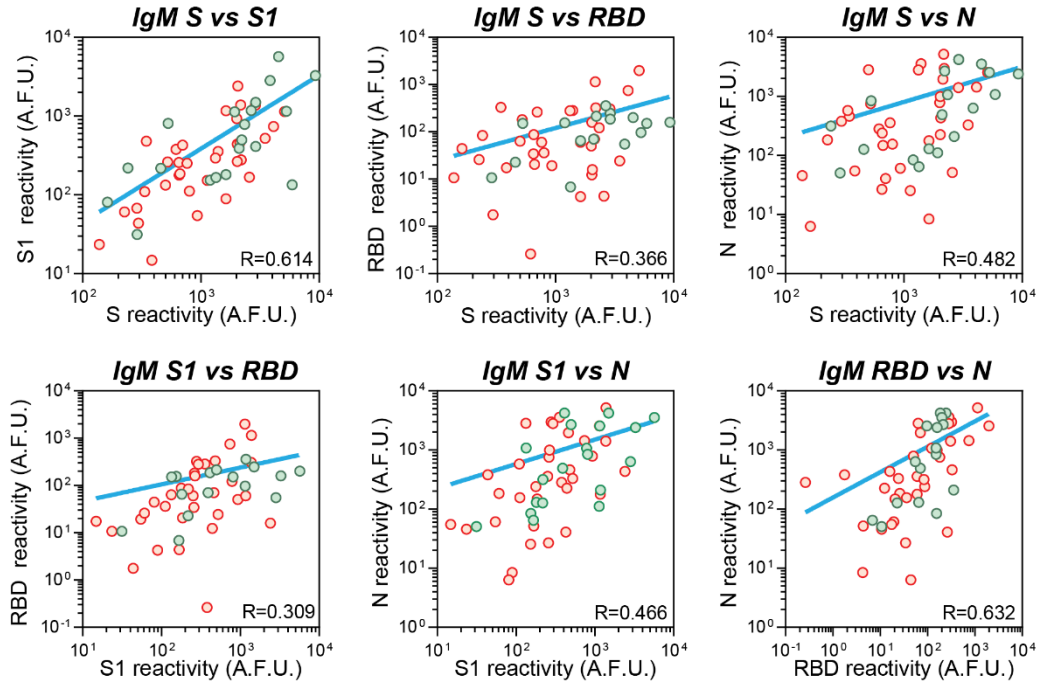
**Figure S1:** Design of the microfluidic device. Red and blue colors denote control and flow layer respectively



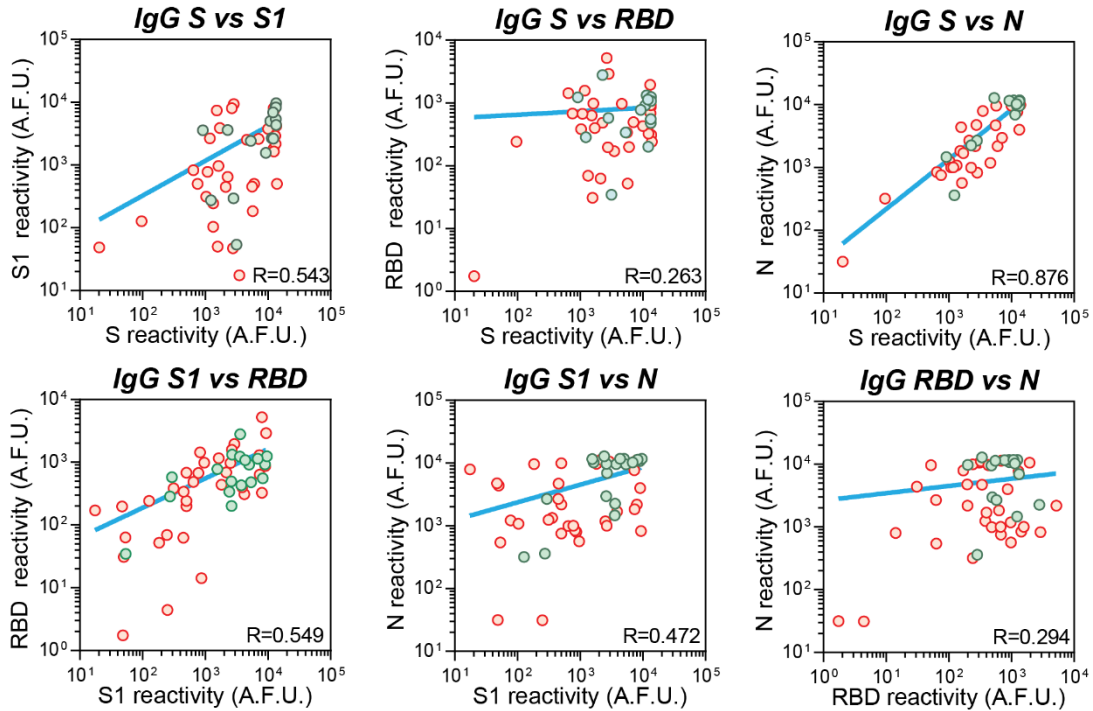
**Figure S2:** Effect of different passivation methods on signal to background ratio (SBR). The blocking solutions used were DyLight650-conjugated NeutraAvidin (NA) at 100  $\mu\text{g}/\text{mL}$ , non-essential amino acids (NEAA) diluted in PBS, and ethanolamine (ETA) at three different concentrations (3, 5 and 10%) diluted in carbonate/bicarbonate buffer and PBS alone as a control.



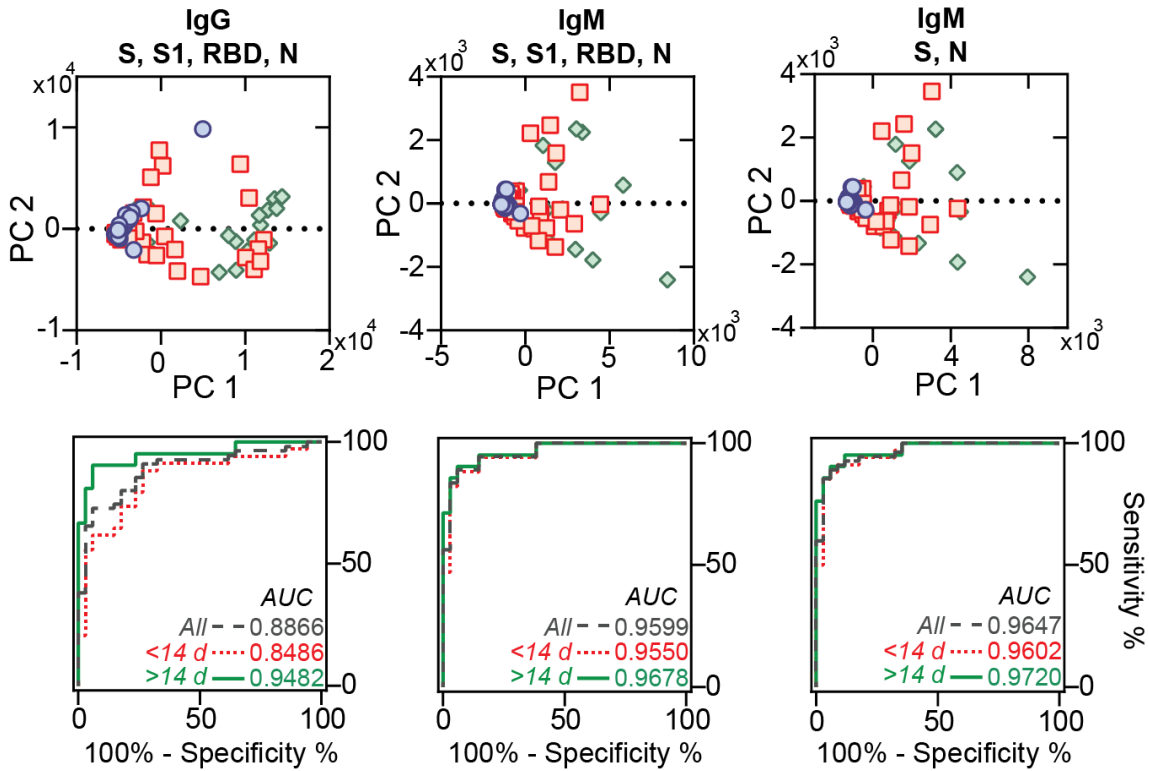
**Figure S3:** IgG and IgM intensities from experiment 1 for the viral antigens Spike, S1, RBD, and NC of both pre-pandemic (blue) and COVID-19 (red) samples when diluted at 1:10, 1:20, 1:25, and 1:50 factor. Bars show mean  $\pm$  s.d. from duplicate experiments.



**Figure S4:** IgM antibody correlations between pairs of antigens. Bottom right corner shows the correlation coefficient.



**Figure S5:** IgG antibody correlations between pairs of antigens. Bottom right corner shows the correlation coefficient.



**Figure S6. Principal component analysis (PCA) of combination of antigens.** Top row shows PCA analysis of immunoglobulins IgG for all the antigens, IgM for all the antigens, and IgM for antigens S and N. Bottom rows shows the ROC for each PC analysis.

**Table S2.** Correlation coefficients between principal components and measured variables, and variance of data explained by each PC.

|                | PC 1  | PC 2  | PC 3  | PC 4  | PC 5  | PC 6  | PC 7  | PC 8  |       |
|----------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| IgG            | Spike | 0.73  | 0.06  | -0.46 | -0.45 | -0.20 | 0.00  | -0.05 | -0.02 |
|                | S1    | 0.27  | 0.86  | 0.12  | 0.33  | 0.20  | -0.11 | 0.13  | 0.02  |
|                | RBD   | 0.04  | 0.15  | 0.11  | 0.07  | -0.09 | 0.75  | -0.62 | -0.01 |
|                | N     | 0.60  | -0.48 | 0.26  | 0.58  | 0.11  | 0.01  | 0.03  | 0.02  |
| IgM            | Spike | 0.15  | 0.06  | 0.76  | -0.37 | -0.33 | -0.32 | -0.22 | -0.06 |
|                | S1    | 0.06  | 0.01  | 0.27  | -0.20 | -0.15 | 0.56  | 0.73  | 0.06  |
|                | RBD   | 0.01  | -0.01 | 0.03  | -0.07 | 0.06  | -0.04 | -0.08 | 0.99  |
|                | N     | 0.10  | -0.08 | 0.18  | -0.41 | 0.88  | 0.08  | -0.05 | -0.10 |
| Variance (%)   | 80.45 | 10.23 | 3.95  | 3.08  | 1.01  | 0.78  | 0.43  | 0.08  |       |
| Cumulative (%) | 80.45 | 90.67 | 94.63 | 97.70 | 98.71 | 99.49 | 99.92 | 100   |       |

**Table S3.** Performance of diagnostic variables for different sample collection times.

| Days post symptom onset | ≥ 14          |               |               |               |               |               |               |               |               |               |               |               | 21 – 30      |
|-------------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|--------------|
| Variable                | Intensity     |               |               |               |               |               |               |               | PC 1 score    |               |               |               | PC 1 score   |
| Antibody isotype        | IgG           | IgM           | IgG           | IgM           | IgG           | IgM           | IgG           | IgM           | IgG/IgM       | IgG           | IgM           | IgM           | IgG          |
| Viral antigen           | S             | S             | S1            | S1            | RBD           | RBD           | N             | N             | All Ag        | All Ag        | All Ag        | S, N          | All Ag       |
| Cut-off                 | 2072          | 240.6         | 2400          | 131.3         | 192.5         | 43.1          | 1219          | 83.4          | -3356         | -3172         | -1052         | -1072         | 5185         |
| Sensitivity (%)         | 85            | 95            | 71.4          | 90.4          | 95.2          | 80.9          | 85.7          | 85.7          | 90.4          | 90.4          | 90.4          | 95.2          | 100          |
| C.I. 95%                | 63.6–<br>96.9 | 76.1–<br>99.8 | 47.4–<br>88.7 | 69.6–<br>98.3 | 76.1–<br>99.8 | 58.0–<br>94.5 | 63.6–<br>96.9 | 63.6–<br>96.9 | 69.6–<br>98.8 | 69.6–<br>98.8 | 69.6–<br>98.8 | 76.1–<br>99.8 | 54.0–<br>100 |
| Specificity (%)         | 94.1          | 91.1          | 94.1          | 82.3          | 82.3          | 97.0          | 97.0          | 73.5          | 94.1          | 94.1          | 94.1          | 88.2          | 100          |
| C.I. 95%                | 80.3–<br>99.3 | 76.3–<br>98.1 | 80.3–<br>99.2 | 65.4–<br>93.2 | 65.4–<br>93.2 | 84.6–<br>99.9 | 84.6–<br>99.9 | 55.6–<br>87.1 | 80.3–<br>99.2 | 80.3–<br>99.2 | 80.3–<br>99.2 | 72.5–<br>96.7 | 89.4–<br>100 |
| PPV at prevalence = 5 % | 43            | 36            | 39            | 21            | 22            | 59            | 60            | 15            | 45            | 45            | 45            | 30            | 100          |
| NPV at prevalence = 5%  | 99            | 100           | 98            | 99            | 100           | 99            | 99            | 99            | 99            | 99            | 99            | 100           | 100          |
| PPV at prevalence = 10% | 62            | 54            | 57            | 36            | 37            | 75            | 76            | 26            | 63            | 63            | 63            | 47            | 100          |
| NPV at prevalence = 10% | 98            | 99            | 97            | 99            | 99            | 98            | 98            | 98            | 99            | 99            | 99            | 99            | 100          |
| PPV at prevalence = 15% | 72            | 65            | 68            | 47            | 49            | 83            | 83            | 36            | 73            | 73            | 73            | 59            | 100          |
| NPV at prevalence = 15% | 97            | 99            | 95            | 98            | 99            | 97            | 97            | 97            | 98            | 98            | 98            | 99            | 100          |
| PPV at prevalence = 20% | 78            | 73            | 75            | 56            | 57            | 87            | 88            | 45            | 79            | 79            | 79            | 67            | 100          |
| NPV at prevalence = 20% | 96            | 99            | 93            | 97            | 99            | 95            | 96            | 95            | 98            | 98            | 98            | 99            | 100          |