**SUPPLEMENTARY APPENDIX.**

# A betacoronavirus multiplex microsphere immunoassay detects early SARS-CoV-2 seroconversion and antibody cross reactions

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## Table S1. MMIA SARS-CoV-2 RBD performance

| | SARS-CoV-2 PCR Status/Archival Sera | | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| SARS-CoV-2 MMIA IgG Antibody Test | Positive | 135 | 0 | 135 |
| | Negative | 20 | 114 | 134 |
| | Total | 155 | 114 | 263 |
| | Sensitivity | 87.1% | | |
| | Specificity | | 100% | |

## Table S2. IgG and IgM seropositivity within 28 days post-symptom onset (dpso)

| dspo | IgG+ | IgG+/IgM+ |
|---|---|---|
| 7 – 14 | 80.0% (12/15) | 73.3% (11/15) |
| 15 – 28 | 100% (31/31) | 93.5% (29/31) |

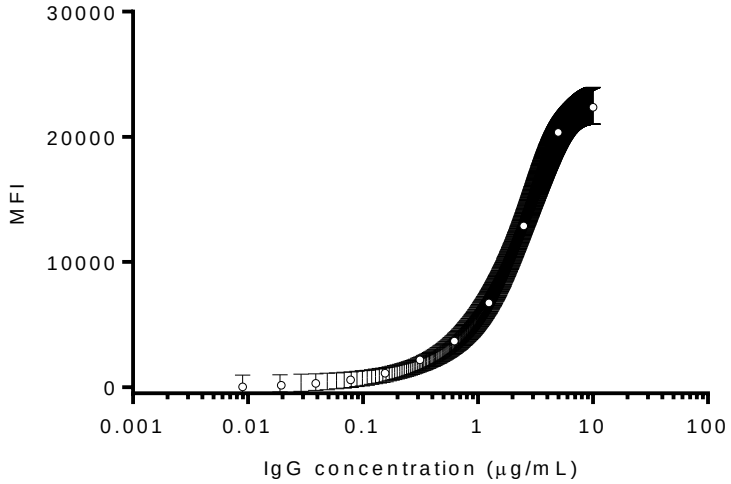


**Figure S1. SARS-CoV-2 IgG detection in experimentally challenged NHP serum samples.** A sigmoidal curve was used to fit the MEAN±SEM of two independent experiments performed in technical triplicates. MFI, median fluorescence intensities.

**A.** **B.**

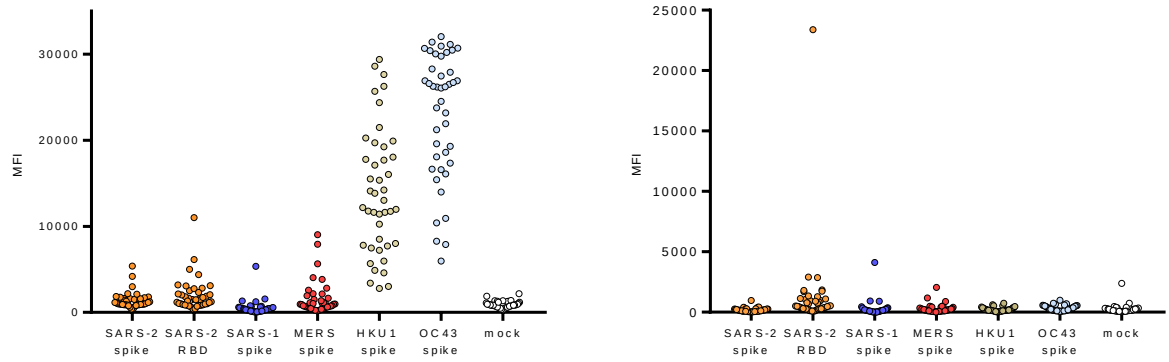


**Figure S1. Establishment of 99.7% probability threshold MFI cutoffs for SARS-CoV-2 positive antibodies.** Convalescent serum samples (n= 43) from HCoV PCR-positive subjects were tested in the β-CoV MMIA to determine **(A)** IgG antibody and **(B)** IgM antibody reactivity to SARS-CoV-2 spike protein and RBD with a pre-2019 sera bank. Data represent the MEAN of three independent experiments performed in technical duplicates.

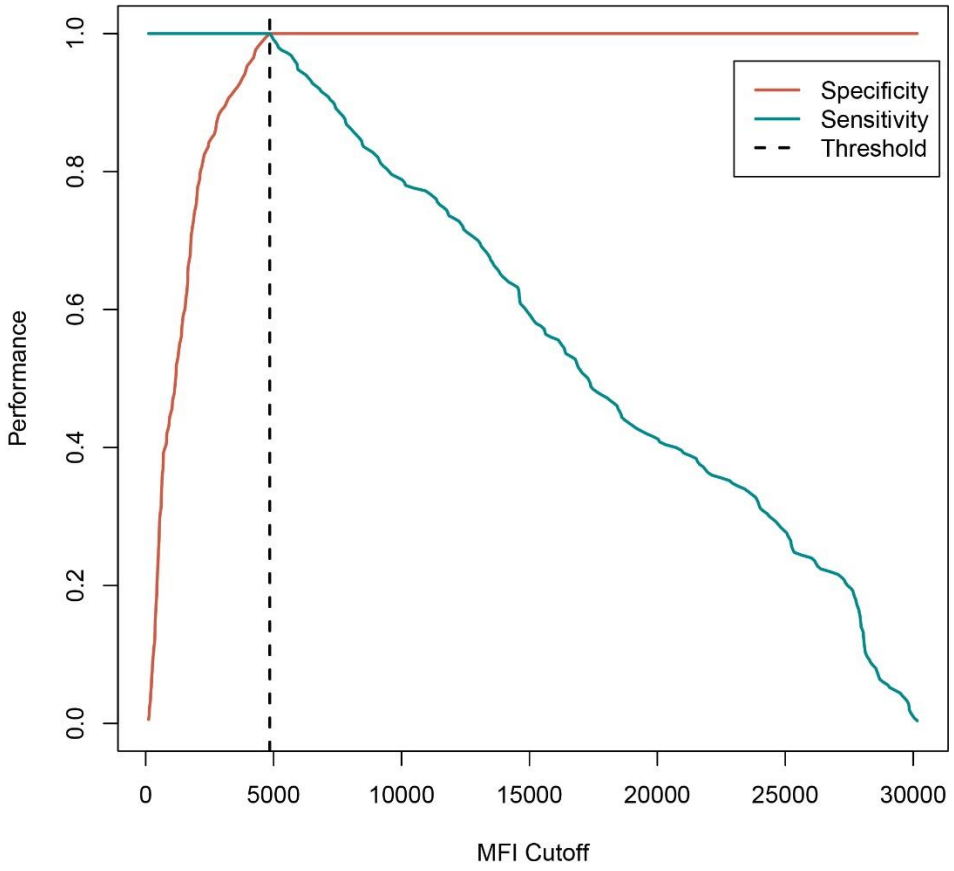


**Figure S3. Receiver operating characteristic curve analysis of SARS-CoV-2 spike protein IgG antibody reactivity in a β-CoV MMIA.** PCR-confirmed SARS-CoV-2 positive and negative serum samples (n= 422) were tested with β-CoV MMIA and 100% was achieved at threshold cutoff of 4854 MFI.

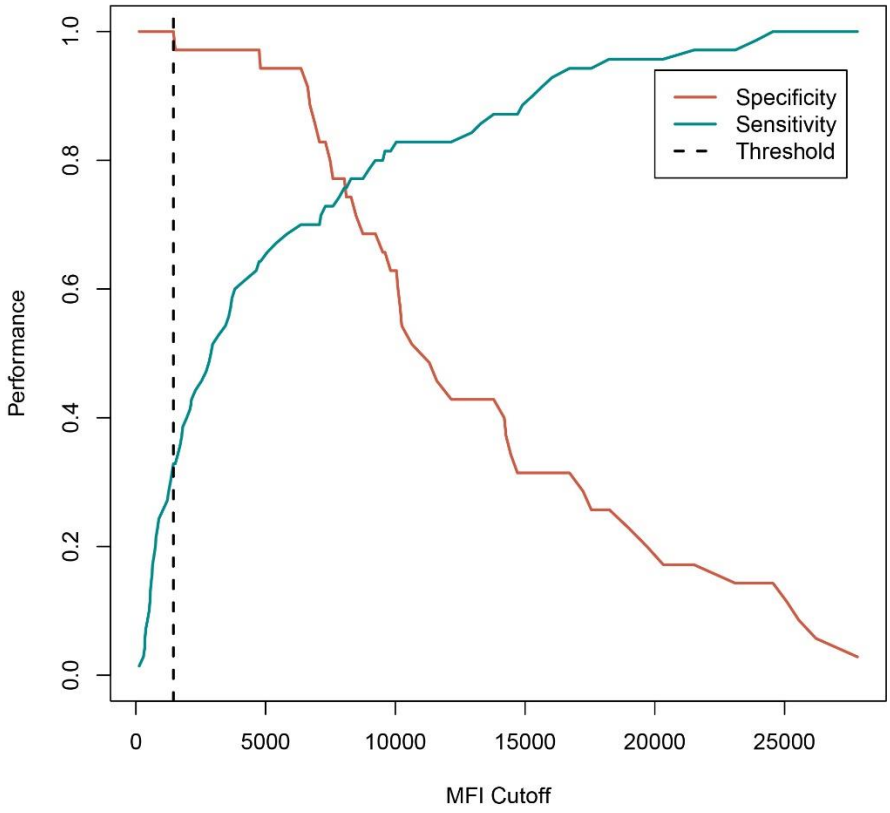


**Figure S4. Receiver operating characteristic curve analysis of SARS-CoV-2 spike protein IgM antibody reactivity in a β-CoV MMIA.** PCR-confirmed SARS-CoV-2 positive and negative serum samples (n= 105) were tested with β-CoV MMIA and 100% specificity was achieved at threshold cutoff of 1446 MFI.

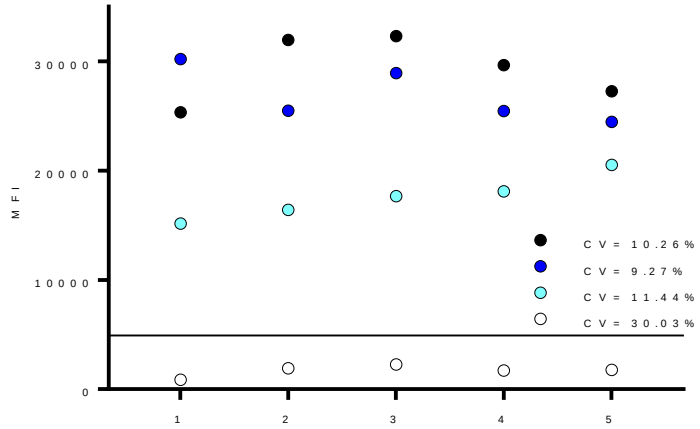


**Figure S5. Positive and negative results are reproducible over independent MMIA tests.** Selected positive(s) and negative serum samples were tested across independent experiments. CV, coefficient of variation, percentages are indicated on the graphs for each sample. A solid line indicates the threshold cutoff for positive IgG.