

Expanding the range of quantified IgG antibodies in a SARS-CoV-2 ELISA assay

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COVID-19
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FACE À LA COVID-19



Lunenfeld-Tanenbaum
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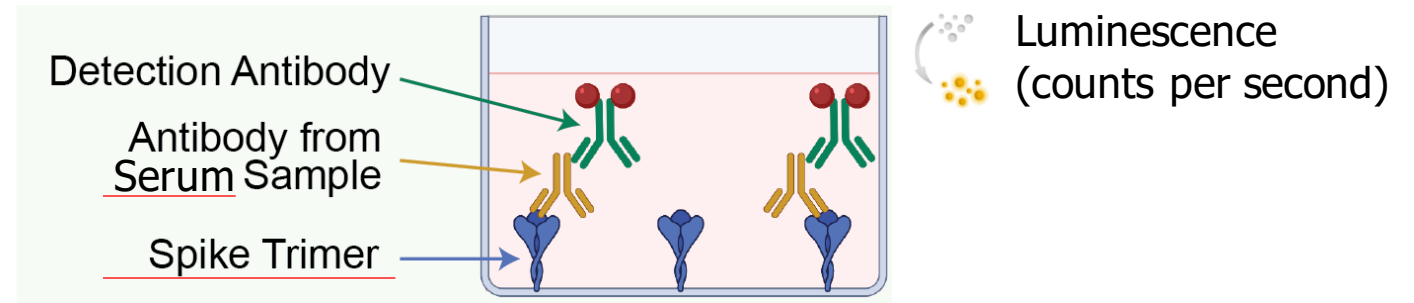
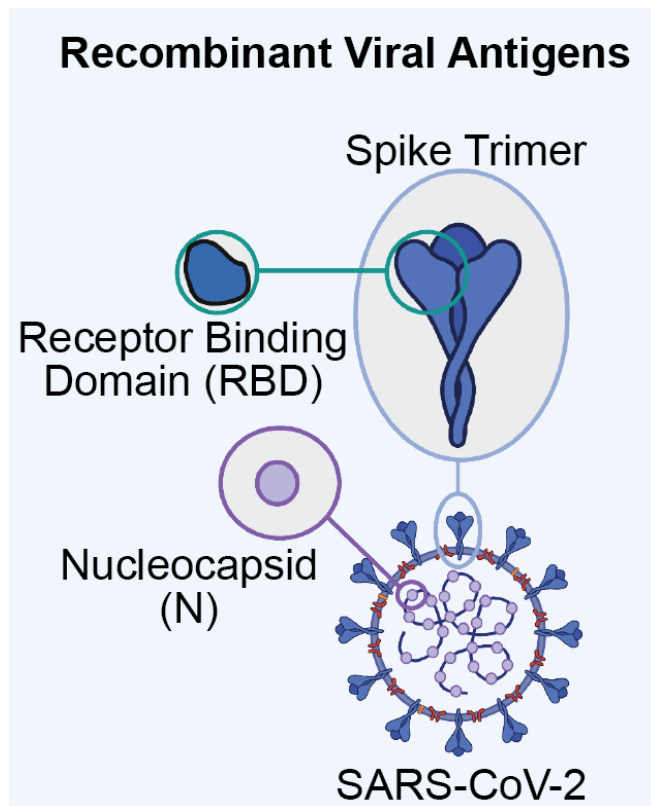
Sinai Health System

Disclaimer

I have no COIs to declare related to this study

Research during the pandemic moves fast

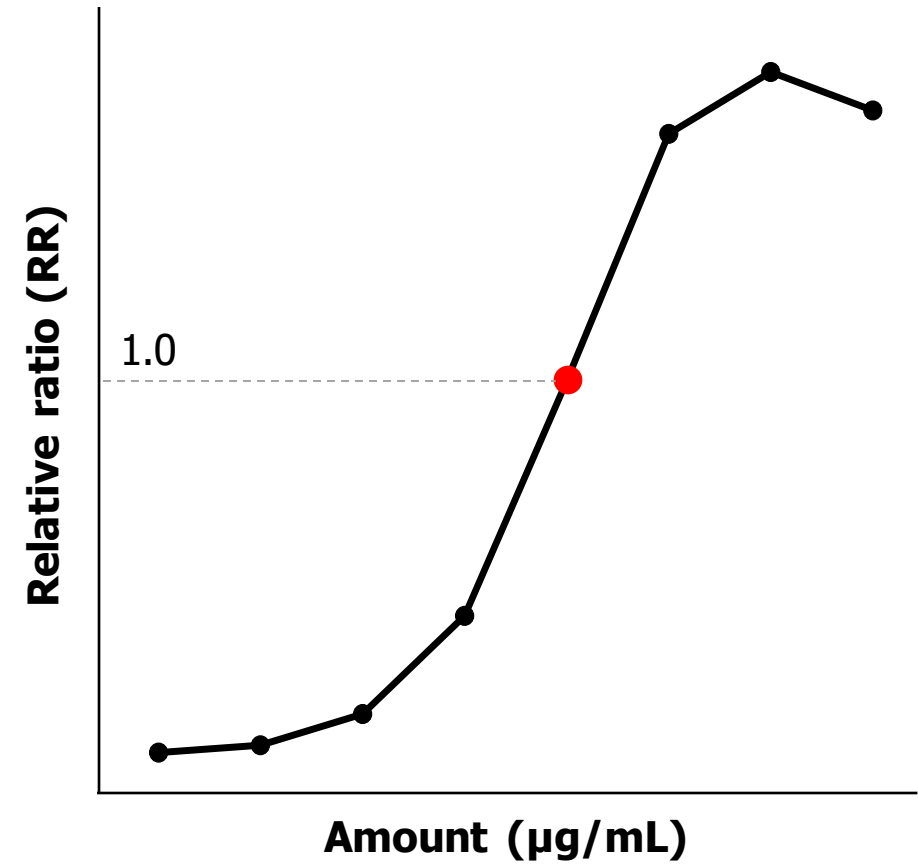
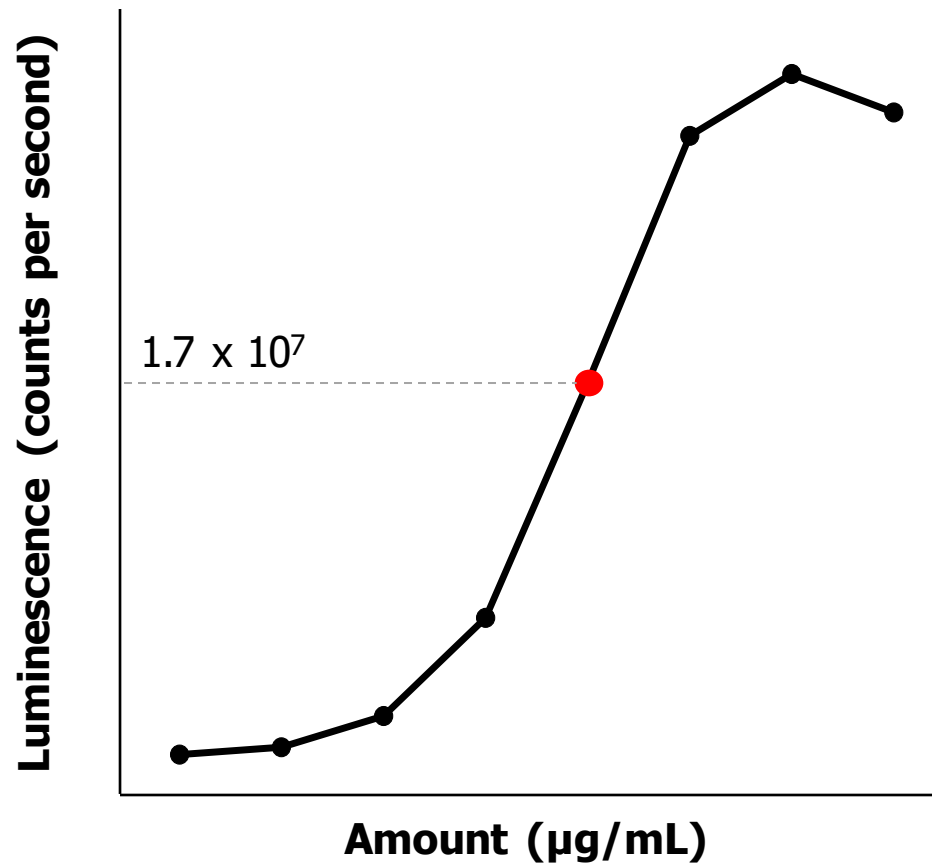
- Our in-house ELISA assay was first developed in March 2020
- In three years, we have processed >185,000 unique samples
- Serology assays need to be dynamic in this rapidly changing landscape



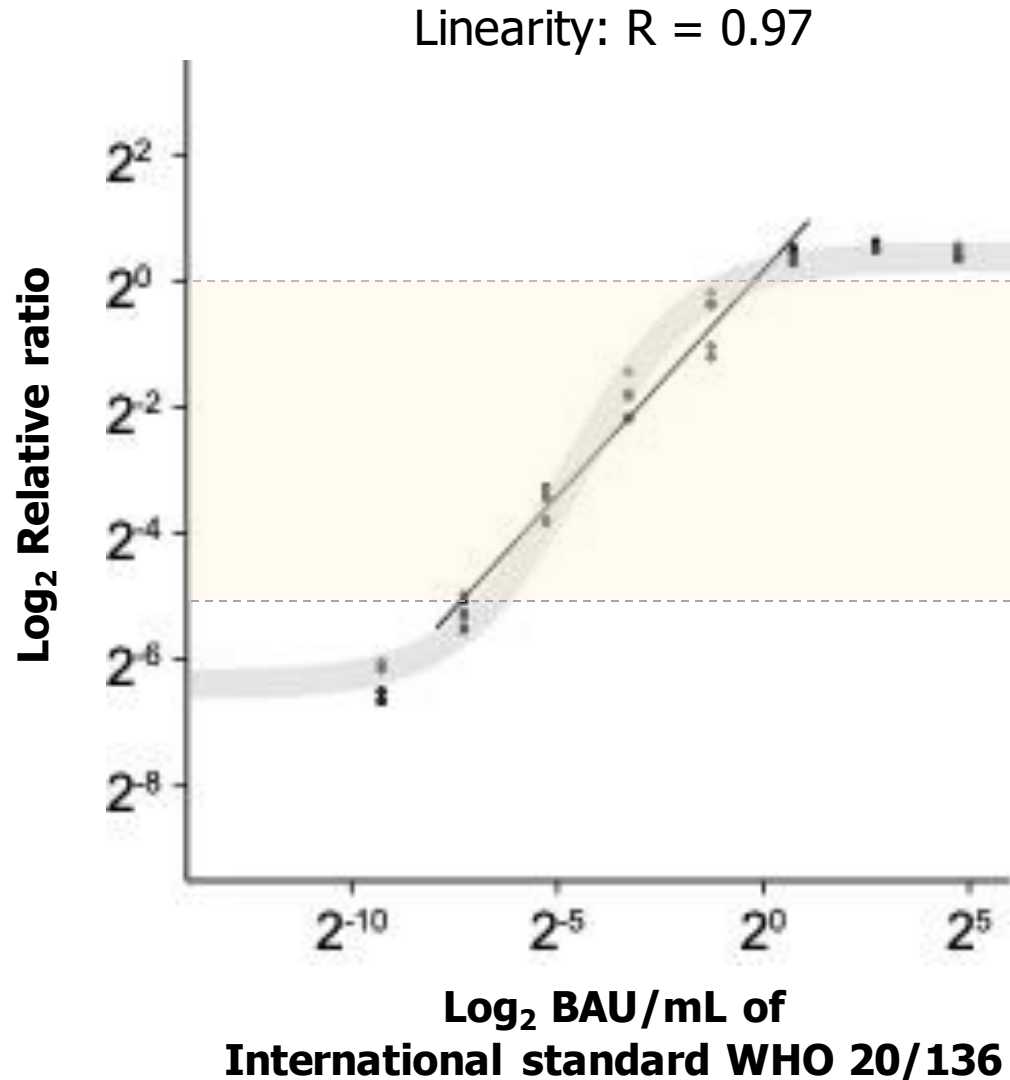
Reagents supplied by the
National Research Council of Canada
Colwill et al., Clin Trans Immunology (2022)

Luminescence signals are first calculated as relative ratios to an internal standard

VHH72 anti-spike antibody standard curve



Relative ratios are then converted to BAU/mL units

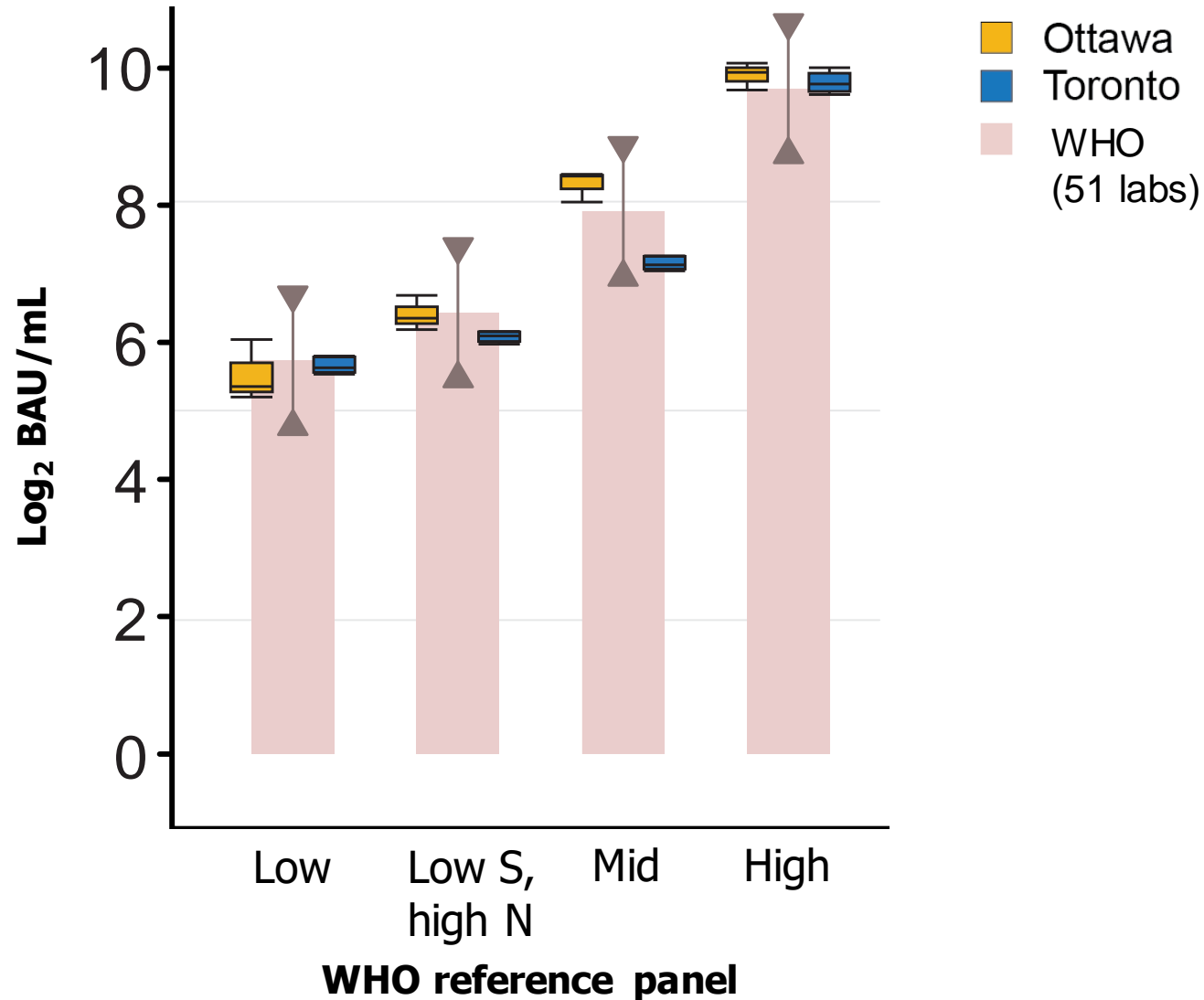


- The WHO international standard consists of pooled plasma from 11 convalescent patients. It has an arbitrary unitage of 1000 BAU/mL
- Calibration to the international standard allows for results to be converted to BAU/mL units and compared across labs
- To convert RR to BAU/mL units:

$\log_2(\text{BAU/mL at sample dilution } d) =$

$$\frac{\log_2(\text{RR}) - 0.604}{0.784} + \log_2(d)$$

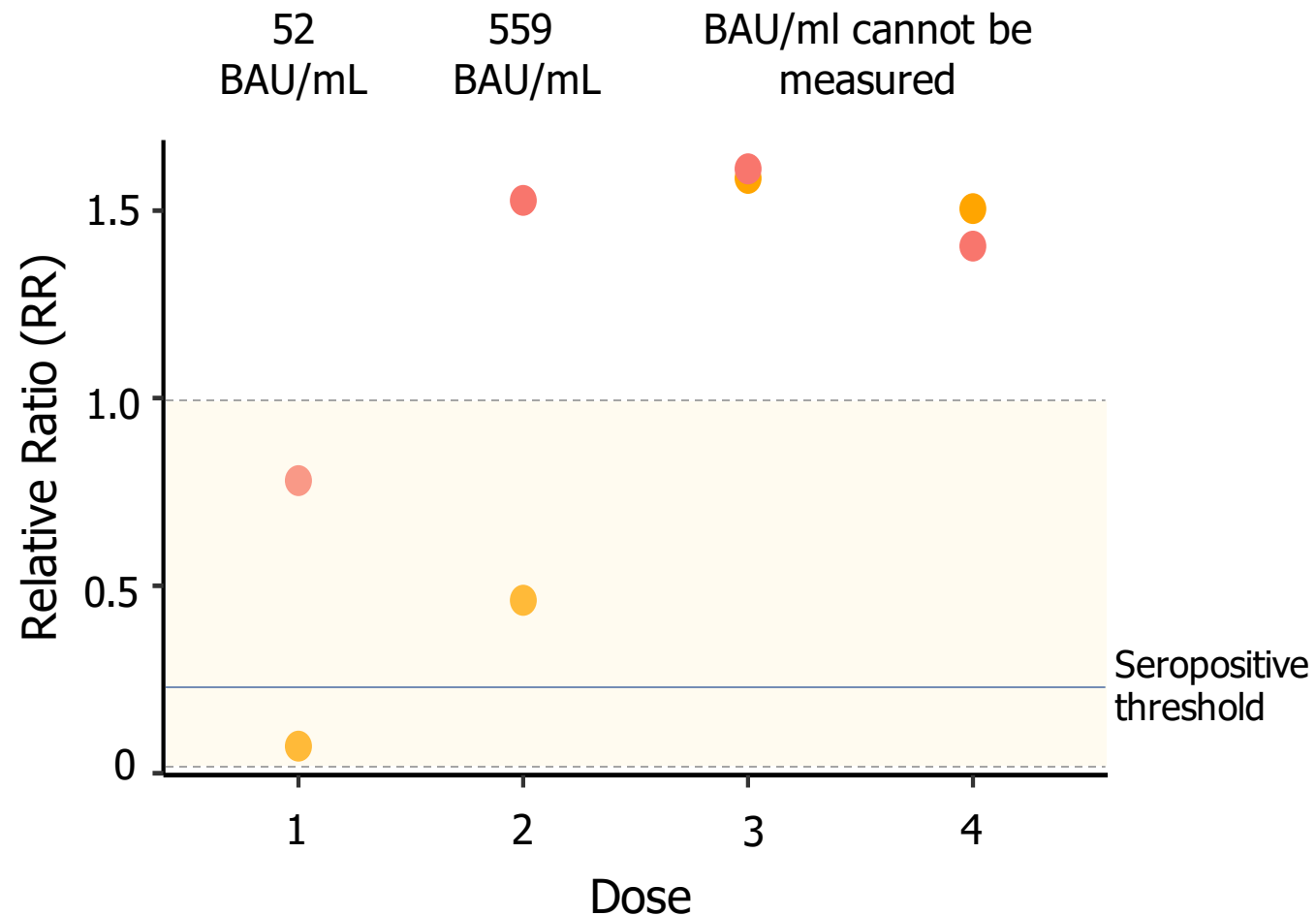
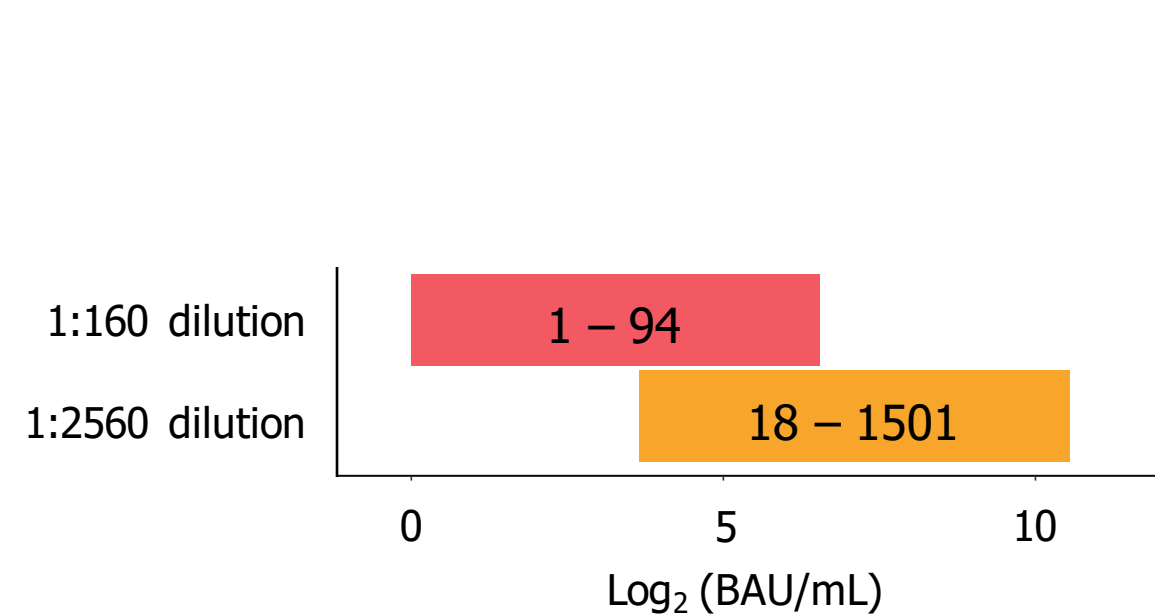
Results from our ELISA assay are similar to the WHO study



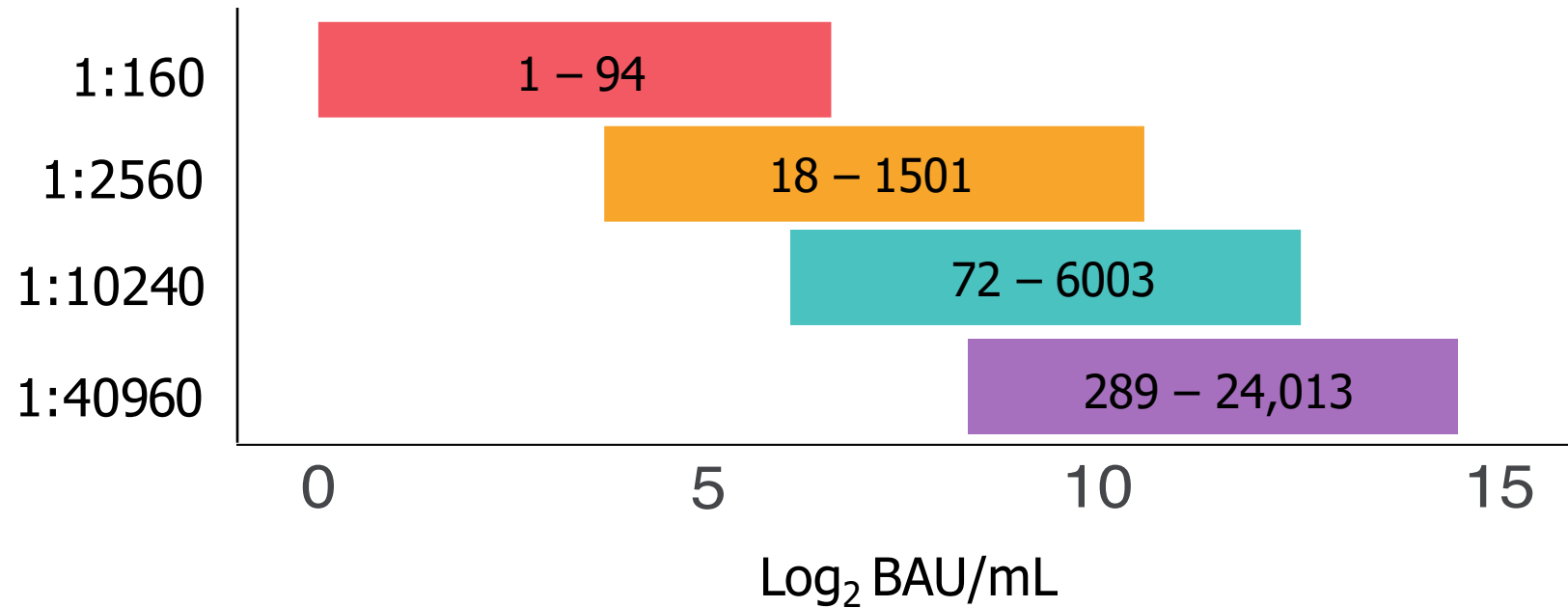
- Our results in BAU/mL are within 0.5 - 2 fold of the geometric mean reported by 51 laboratories in the WHO study
- This gives us confidence in our assay and our BAU/mL conversion formula

Samples with high antibody levels are not measured accurately using previously established dilutions

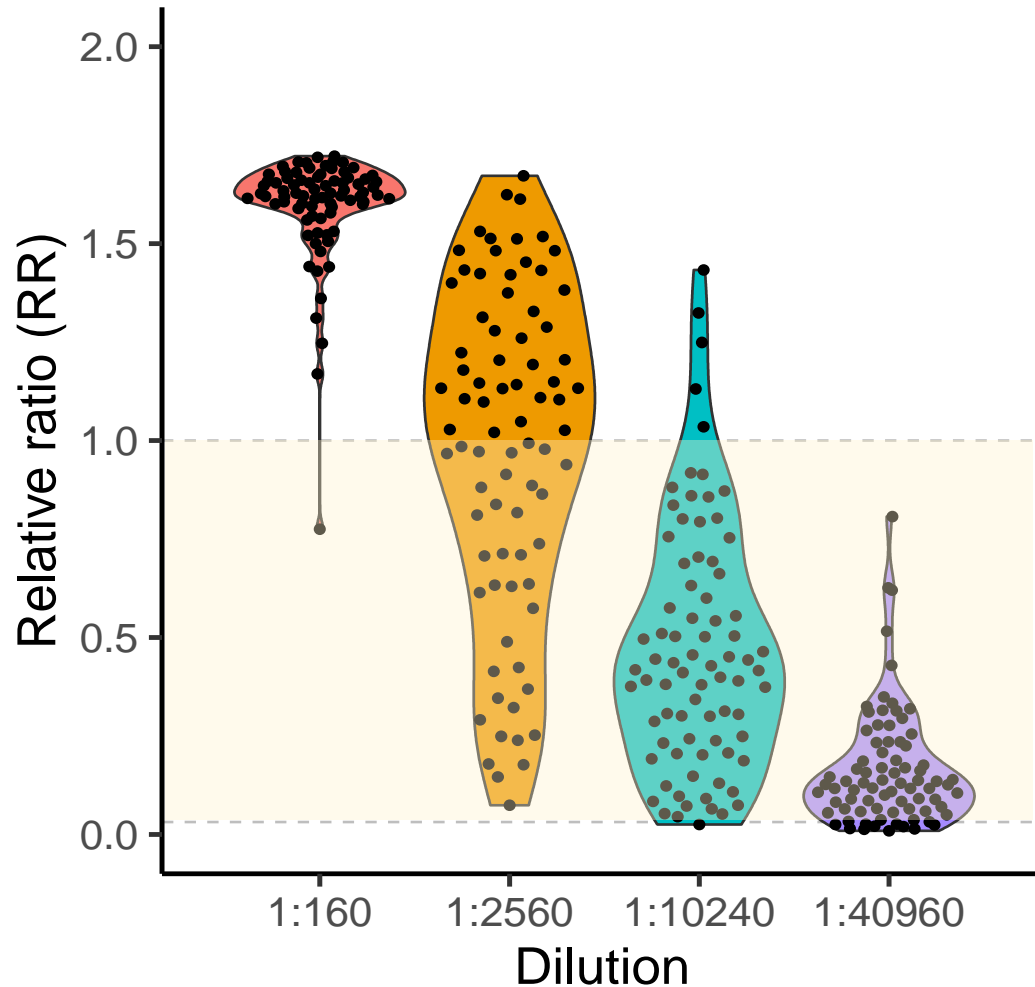
- The dilutions initially selected are 1:160 and 1:2560



Additional sample dilutions expand range of quantitation



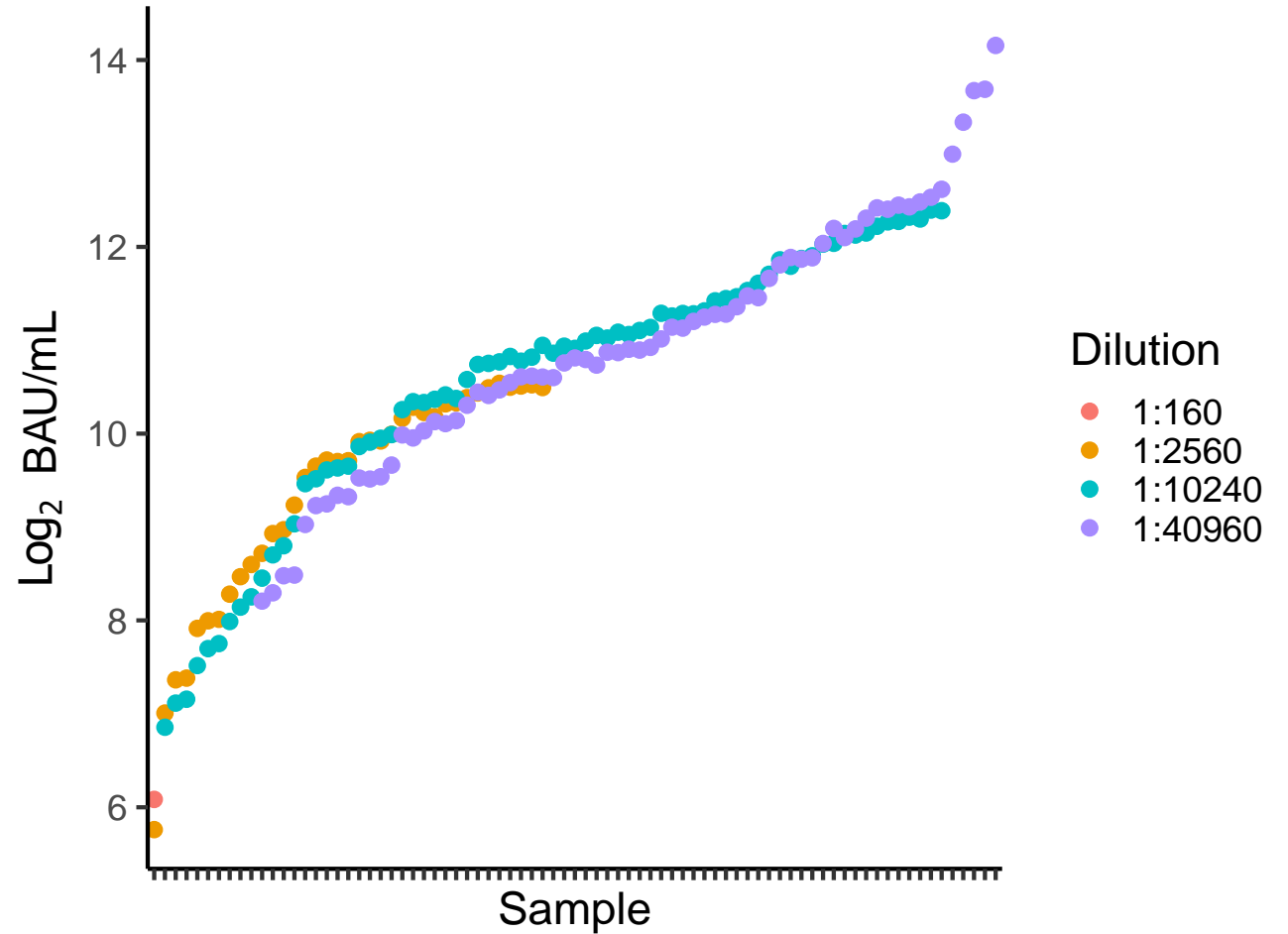
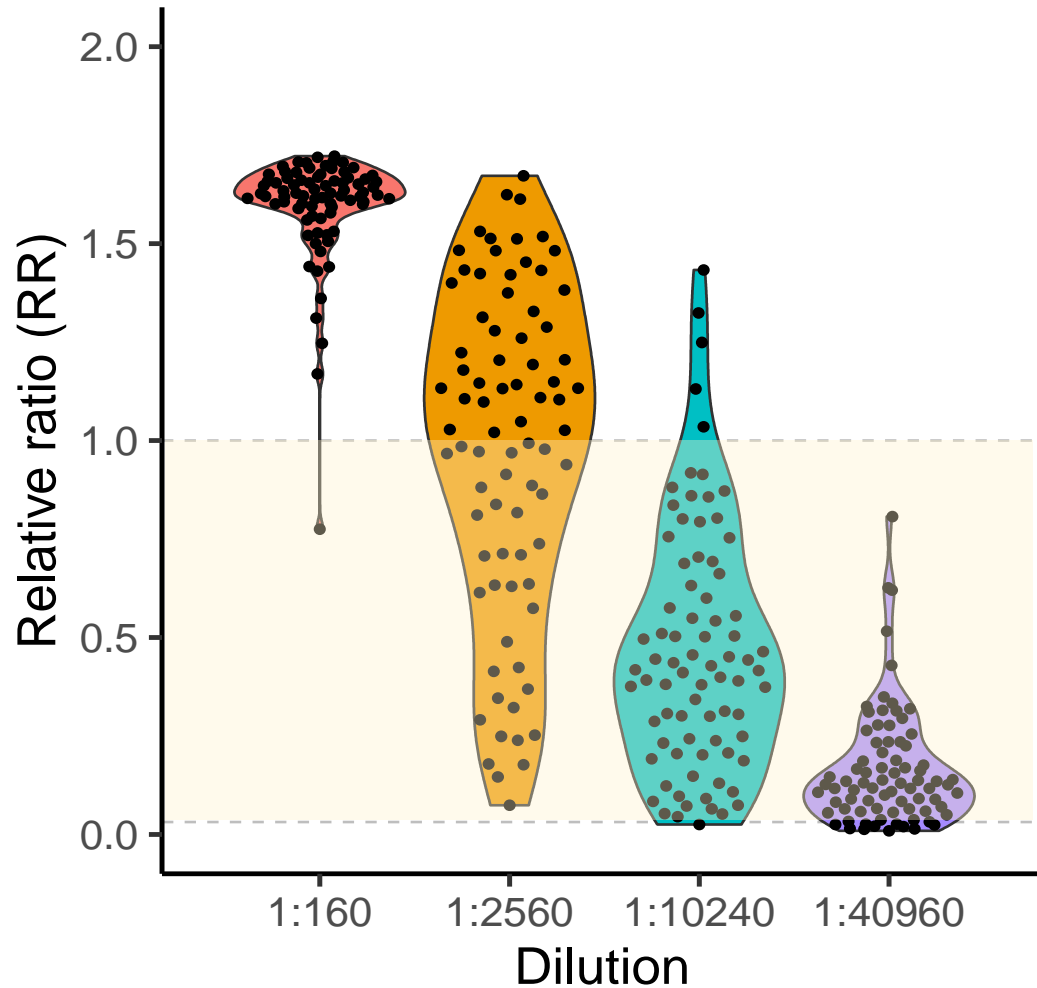
Additional 1:10240 and 1:40960 dilutions allow for BAU/mL to be calculated in saturated samples



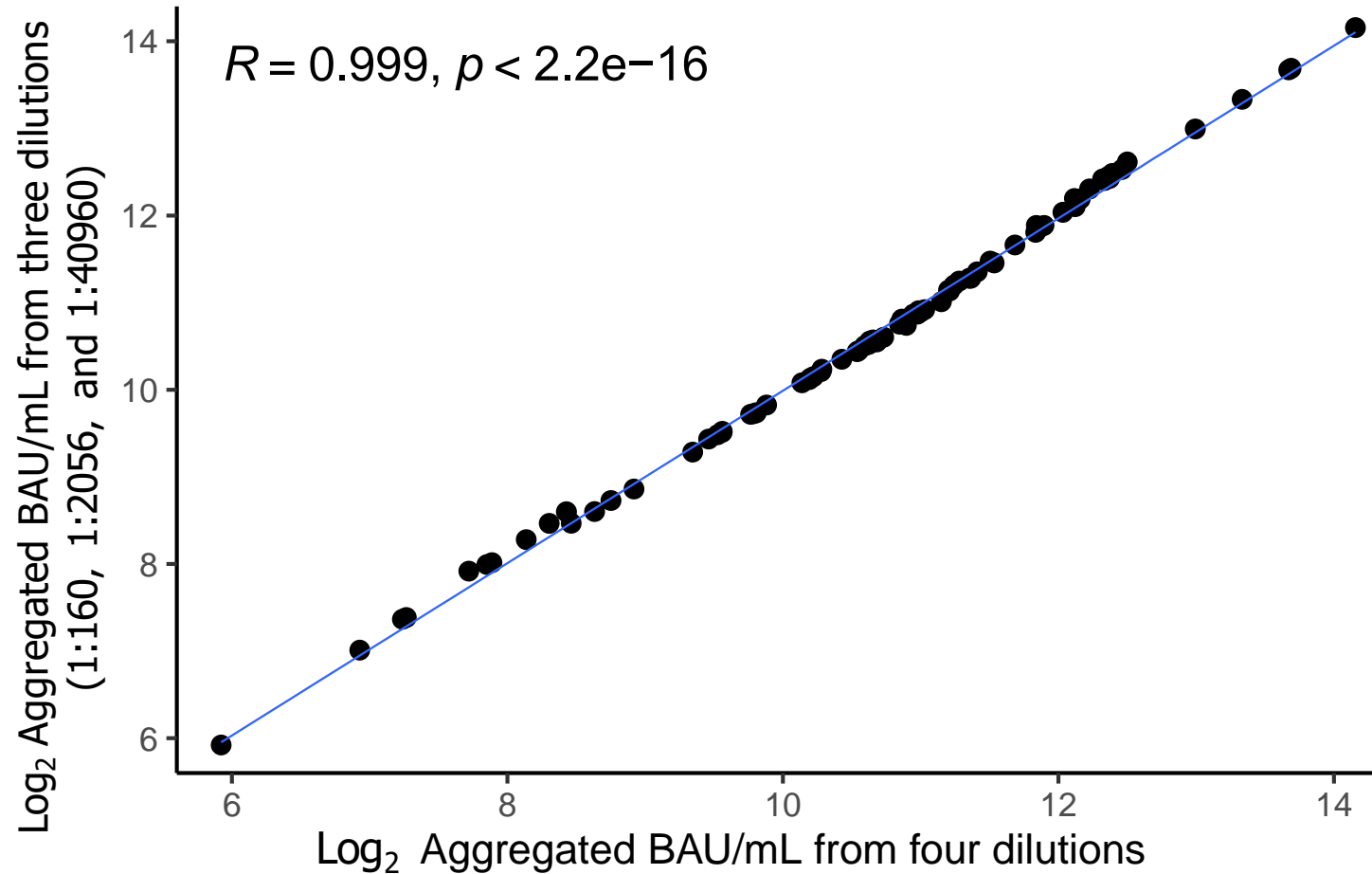
Of 79 samples collected from fully vaccinated and/or infected individuals:

- 42 samples **cannot** be accurately measured in BAU/mL units using 1:160 and 1:2560 dilutions only
- All 79 samples **can** be accurately measured using all four dilutions

BAU/mL values correlate well between dilutions

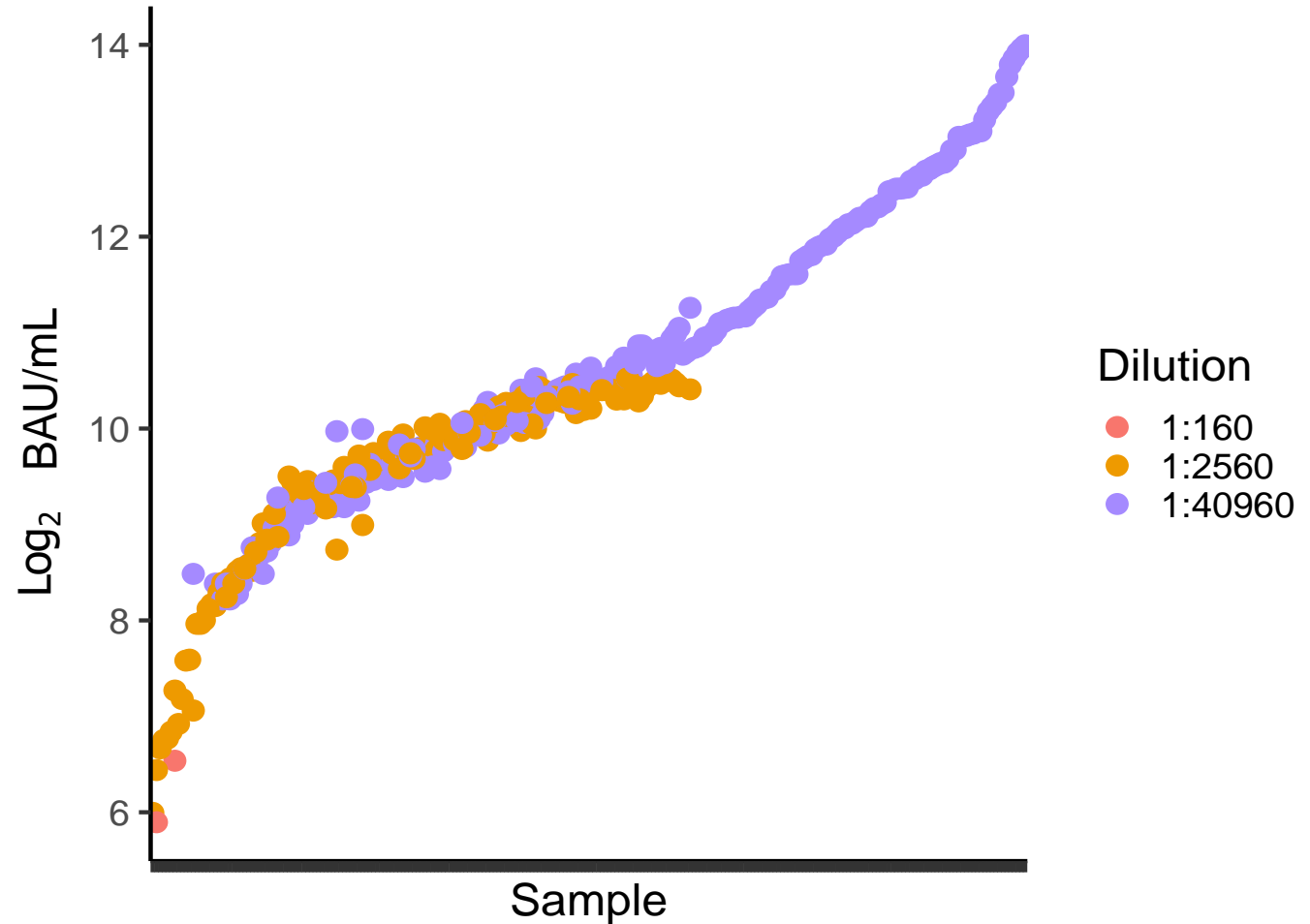
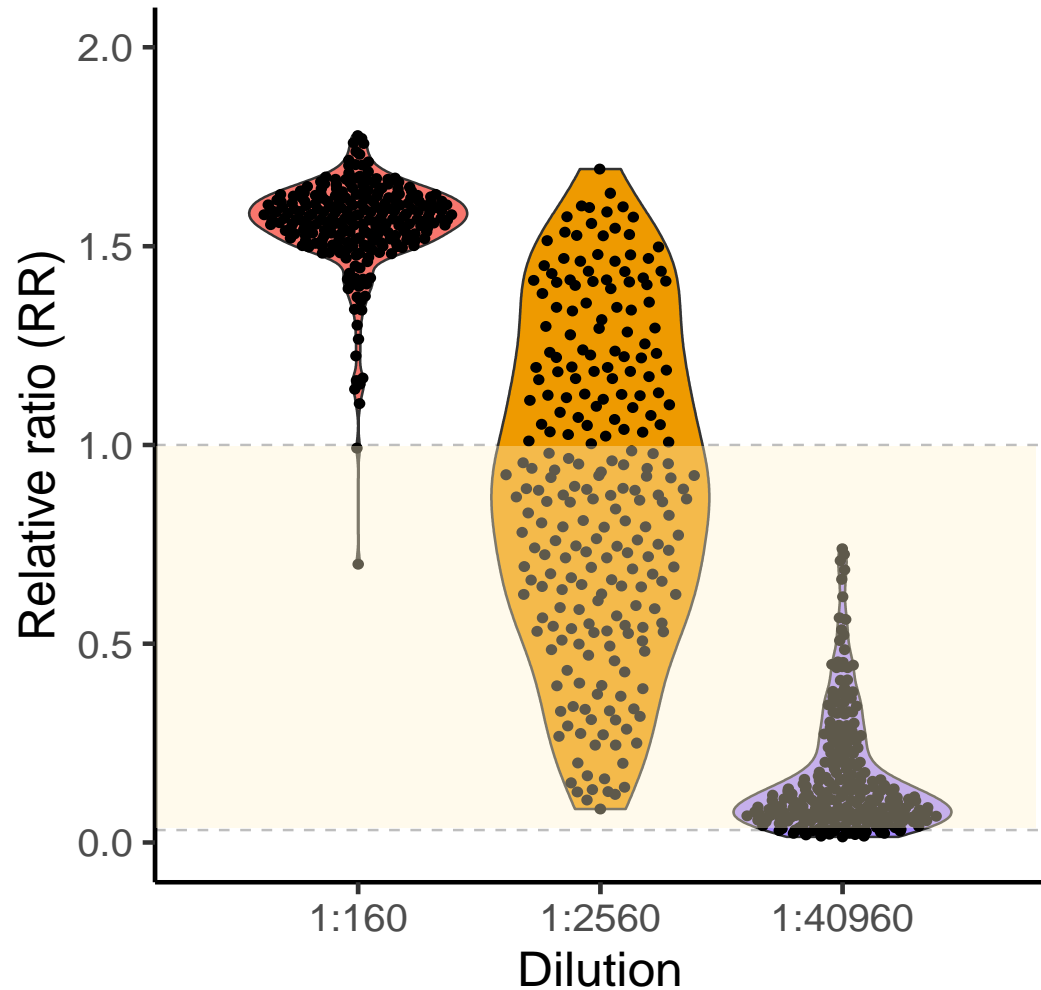


Aggregated BAU/mL values between the three and four dilutions are strongly correlated



- To decrease cost and increase throughput, the 1:10240 dilution does not need to be performed

Testing of an additional 238 samples confirmed that three dilutions are sufficient for conversion to BAU/mL units



Conclusions

- Adding a 1:40960 dilution expands our range of quantitation allowing for previously saturated values to be accurately quantified and reported in BAU/mL units
- Antibody levels can be quantified up to 24013 BAU/mL for S, 71263 BAU/mL for RBD, and 85000 BAU/mL for N

Lessons learned

- In the ever-changing COVID-19 landscape, serological assays need to be dynamic and its parameters may need to be continually reviewed
- Performing three dilutions for each sample is more time-consuming and costly than performing two dilutions. It could not have been implemented at the peak of the pandemic when we had thousands of samples to test
- It may now be implemented for studies that require exact BAU/mL values

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