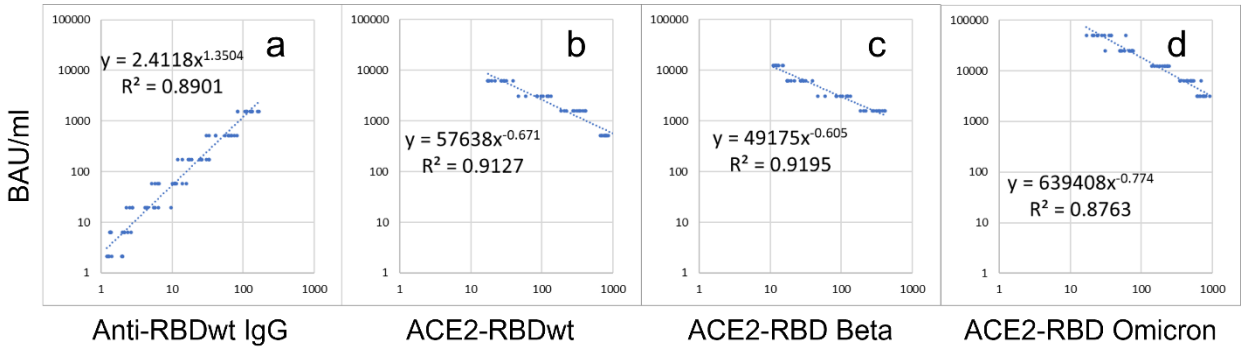
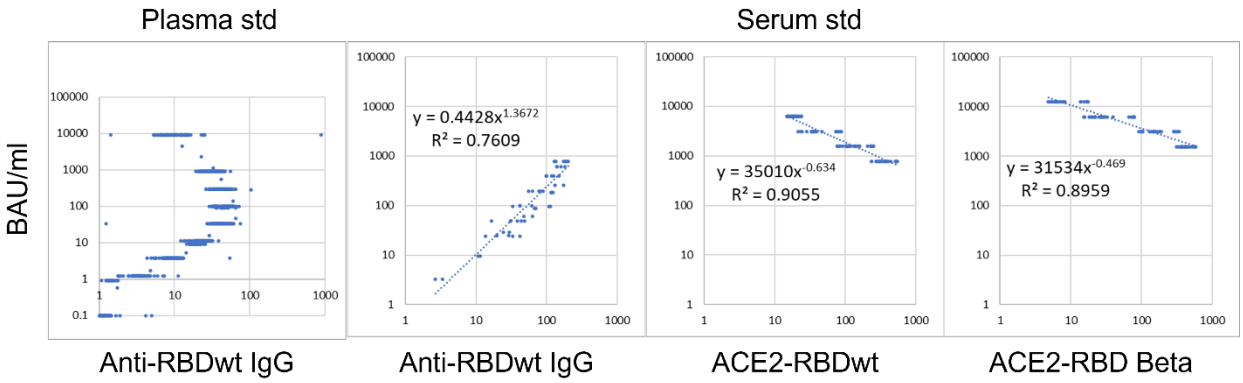
Conversion of signal values measured by Multi-IgG-ACE2-RBD to BAU/ml.




The x- and y axes show normalized signal values measured with Multi-IgG-ACE2-RBD, and BAU/ml as calculated from serial dilution of a standard series, respectively. The results represent all standards measured in the period of February 14 to March 3 2022. The standard series was prepared from a serum determined to contain 53.000 BAU/ml with the Roche anti-SARS-CoV-2 S assay. Normalization was performed by dividing signals measured for standards in each 384 well plate by the mean measured for samples with no detectable anti-RBDwt IgG. **a:** anti-RBD-wt rMFI. **b**: binding of ACE2 to RBD-wt, **c:** binding of ACE2 to RBD-beta, **d:** binding of ACE2 to RBD Omicron. Excel was used to generate regression formulas to convert signals measured for test samples measured in each experiment to BAU/ml. Excel (IF(AND) functions were used to select the value from the correct parameter. The best curve fit was obtained using the “power function”. This is equivalent to performing linear regression with log-transformed data. The data in each scatter plot were filtered on the optimal dynamic range (BAU/ml) for each parameter. Thus, values calculated on basis of RBD-ACE2 interactions were only selected when the anti-RBDwt signal was higher than 5 and the inhibition of RBD-ACE2 interactions was at least 50% (i.e. normalized signal <500). The value for Omicron was used when all RBD-ACE2 interactions were inhibited by at least 50%, while the value for Beta was used when inhibition of ACE2-binding to RBDwt was also at least 50%. The value for ACE2-RBDwt was used when the value was higher than that calculated from the anti-RBDwt signal. For samples with RBD-ACE2 inhibition lower than 50%, the algorithm estimated BAU/ml on basis of the anti-RBDwt signal, and values higher than 2000 were imputed by 2000. Source data: supplementary Table 1.

Conversion of signal values measured by Multi-IgG-ACE2-RBD to BAU/ml (**For Fig. 8**).




The samples in Fig. 8 in the article were analyzed in the period of mid-July to mid-December 2021. At that time, the serum was diluted 1:100 for detection of anti-RBDwt IgG as well as for measurement of RBD-ACE2 interactions. This dilution was chosen to optimize sensitivity in samples obtained from patients on immunosuppressive therapy. In the same period, we used a plasma standard selected to be similar to the WHO international standard (i.e. titer = 1000 BAU/ml,). However, plasma yielded low resolution for anti-RBDwt IgG due to an extensive “hook effect” (see “Plasma std, left dot plot). The reactivity patterns in RBD-ACE2 interaction assays also deviated somewhat from those observed with serum (not shown). We therefore analyzed the serum standard obtained in November 2021 in seven experiments during November and December 2021 at a dilution of 1:100. The results from all experiments were used as input to generate an “average” regression formula for conversion of signals measured for anti-RBDwt sera in 2021 to BAU/ml. To generate formulas for RBD-ACE2 interactions we used results from 11 experiments performed in late December 2021 to mid January 2022. The values reported for BAU/ml in Fig. 8 in the article are therefore an approximation. However, minor variations in absolute titers should be interpreted in view of results from clinical trials showing that a ten-fold increase in neutralizing titers is required for a 10% absolute increase in vaccine efficacy. The classification on basis of neutralizing activity used in Fig. 8 is independent of the standard and therefore more robust.