Supplemental Online Content

Bates TA, McBride SK, Winders B, et al. Antibody Response and Variant Cross-Neutralization After SARS-CoV-2 Breakthrough Infection. *JAMA.* Published online December 16, 2021. doi:10.1001/jama.2021.22898

eMethods

This supplemental material has been provided by the authors to give readers additional information about their work.

Cohort serum collection:

Among fully vaccinated participants with breakthrough infections, after recovery, whole blood (4-6 mL) was collected with a BD Vacutainer® Plus Plastic Serum Tube and centrifuged for 10 minutes at 1000xg. Serum samples were stored at -20°C. Full vaccination was defined as having received 2 doses of BNT162b2 or mRNA-1273, or 1 dose of Ad26.COV2.S.

SARS-CoV-2 variants sequencing

SARS CoV-2 testing was performed as previously described.[1] Briefly, RNA extraction was performed using one of three methods (Maxwell RSC, MagNA Pure 96, or KingFisher Flex) according to manufacturer instructions using kit viral transport starting volumes of 300 µL, 200 µL or 200 µL, respectively. PCR tests were considered valid if internal control RNA (RNase P or MS2) was detected. PCR was validated to a lower limit of detection of ~ 5 genomic copies/reaction using known standards. Valid tests were interpreted as detected when 2 or 3 viral targets were reactive, inconclusive if a single viral target was reactive and otherwise negative.

SARS CoV-2 genomic sequencing was performed using the Ion AmpliSeq™ SARS-COV-2 Insight Research Panel Assay according to manufacturer instructions with residual RNA from SARS-COV-2 testing. Reverse transcription was performed using the Ion Torrent™ NGS Reverse Transcription Kit. Sequence data were analyzed and aligned using plugins GenerateConsensus to generate FASTA files and SARS-CoV-2

Coverage Analysis for coverage depth. FASTA files were manually reviewed and uploaded into GISAID and NCBI.

Enzyme-linked immunosorbent assays (ELISA) and Focus reduction neutralization tests (FRNT)

ELISAs were performed as previously described.[2] The following proteins were used: SARS-CoV-2 RBD produced in Expi293F cells as described[3], N (SARS-CoV-2 Nucleocapsid-His, insect cell-expressed, SinoBio Cat: 40588-V08B, Item #NR-53797, lot #MF14DE1611). FRNT assays were carried out as previously described.[3] Duplicate 5x4.7-fold (1:10-1:4879) serial dilutions of participant sera were prepared in 96-well plates.

SARS-CoV-2 and variant isolates

Viral stocks were propagated in Vero E6 cells as previously described.[3] The following SARS-CoV-2 isolates were used: USA-WA1/2020 [lineage A] (NR-52281), USA/CA_CDC_5574/2020 [lineage B.1.1.7 – alpha] (NR-54011), hCoV-19/South Africa/KRISP-K005325/2020 [lineage B.1.351 – beta] (NR-54009), hCoV-19/Japan/TY7-503/2021 [lineage P.1 – gamma] (NR-54982), and hCoV-19/USA/PHC658/2021 [lineage B.1.617.2 – delta] (NR-55611) were obtained from BEI Resources.

Statistical analysis

$FRNT_{50}$ and EC_{50} values were calculated by fitting to a dose-response curve as previously described.[3] Final $FRNT_{50}$ values below the limit of detection (1:20) were set to 1:19. Final EC_{50} values below the limit of detection of 1:25 for N, Spike RBD, IgG, IgA

were set to 1:24 and 1:12.5 for IgM was set to 1:12. Individuals for whom a breakthrough variant could not be determined were excluded from the delta potency analysis.

References

1. Fan, G., Qin, X., Streblow, D. N., Hoyos, C. M. & Hansel, D. E. Comparison of SARS-CoV-2 PCR-Based Detection Using Saliva or Nasopharyngeal Swab Specimens in Asymptomatic Populations. *Microbiol Spectr* **9**, e0006221 (2021).

2. Bates, T. A. *et al.* Age-Dependent Neutralization of SARS-CoV-2 and P.1 Variant by Vaccine Immune Serum Samples. *JAMA* (2021) doi:10.1001/jama.2021.11656.

3. Bates, T. A. *et al.* Neutralization of SARS-CoV-2 variants by convalescent and BNT162b2 vaccinated serum. *Nat Commun* **12**, 5135 (2021).