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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: S Jangra, C Ye, R Rathnasinghe, et al. SARS-CoV-2 spike E484K mutation reduces antibody neutralisation. *Lancet Microbe* 2021; published online April 7. https://doi.org/10.1016/S2666-5247(21)00068-9.

Table 1: Description of serum samples obtained from human subjects

	SERUM				
	Seropositive, vaccine	Spike IgG response	Sex	Age group (yrs)	Days post 1 vaccine dose (Pfizer)
	V1	Strong positive	F	>60	68
	V2	Strong positive	M	30-40	47
	V3	Strong positive	F	50-60	47
	V4	Strong positive	F	40-50	49
	V5	Strong positive	F	30-40	48
	Seropositive, infection	Spike IgG response	Sex	Age group (yrs)	Days post onset of symptoms
	P1	Weak positive	M	20-29	260
	P2	Weak positive	M	50-59	NA
	P3	Weak positive	F	30-39	111
	P4	Weak positive	F	30-39	221
	P5	Weak positive	F	30-39	254
	P6	Weak positive	F	20-29	247
	P7	Weak positive	M	30-39	220
	P8	Weak positive	F	20-29	Asymptomatic
	P9	Moderate positive	M	30-39	NA
	P10	Moderate positive	F	30-39	197
	P11	Moderate positive	F	50-59	Asymptomatic
	P12	Moderate positive	F	30-39	Asymptomatic
	P13	Moderate positive	M	30-39	234
	P14	Moderate positive	F	20-29	273
	P15	Moderate positive	M	30-39	Asymptomatic
	P16	Moderate positive	F	20-29	258
	P17	Moderate positive	F	20-29	246
	P18	Moderate positive	M	20-29	Asymptomatic
	P19	Moderate positive	F	50-59	204
	P20	Strong positive	F	50-59	NA
	P21	Strong positive	F	30-39	245
	P22	Strong positive	M	NA	170
	P23	Strong positive	F	>60	Asymptomatic
	P24	Strong positive	F	40-49	NA
	P25	Strong positive	F	50-59	191
	P26	Strong positive	F	30-39	NA
	P27	Strong positive	F	50-59	113
	P28	Strong positive	M	>60	Asymptomatic
	P29	Strong positive	M	18-19	218
	P30	Strong positive	M	50-59	219
	Seronegative, post pandemic	Spike IgG response	Sex	Age group (yrs)	Days from last negative serology
	N1	Negative	F	40-50	23
	N2	Negative	F	20-29	24
	N3	Negative	F	20-29	23
	N4	Negative	F	30-35	22

Methods section:

50% tissue culture infective dose ($TCID_{50}$) calculation and *in vitro* microneutralization assay:

To estimate the neutralizing efficiency of human sera, *in vitro* microneutralization assays were performed. Human sera were inactivated at 56°C for 30 min. Serum samples were serially diluted 3-fold starting from 1:30 dilution in Vero-E6-infection medium (DMEM+ 2% FBS+ 1% non-essential amino acids). The samples were incubated with 450 tissue culture infective dose 50 ($TCID_{50}$) of either USA-WA1/2020 or rSARS-CoV-2 E484K for 1 hour in an incubator at 37°C, 5% CO_2 followed by incubation with pre-seeded Vero-E6 at 37°C for 48 hours. The plates were fixed in 4% formaldehyde at 4°C overnight. For $TCID_{50}$ calculation, the virus stock was serially diluted 10-fold starting with 1:10 dilution and incubated on Vero-E6 cells for 48 hours followed by fixation in 4% Formaldehyde. The cells were washed with 1xPBS and permeabilized with 0.1% Triton X-100 in 1XPBS. The cells were washed again and blocked in 5% non-fat milk in 1xPBS+ 0.1% Tween-20 for 1 hour at room temperature. After blocking, the cells were incubated with anti-SARS-CoV-2 NP and anti-spike monoclonal antibodies, mixed in 1:1 ratio, for 1.5 hours at room temperature. The cells were washed in 1xPBS and incubated with 1:5000 diluted HRP-conjugated anti-mouse IgG secondary antibody for 1 hour at RT followed by a brief PBS wash. Finally, 100µl tetramethyl benzidine (TMB) substrate was added and incubated at RT until blue color appeared, and the reaction was terminated with 50µl 1M H_2SO_4. Absorbance was recorded at 450nm and 650nm and percentage reduction in infection was calculated as compared to negative control. We performed all experiments in a blinded manner.

Serum samples from human subjects:

The study protocols for the collection of clinical specimens from individuals with and without SARS-CoV-2 infection by the Personalized Virology Initiative were reviewed and approved by the Mount Sinai Hospital Institutional Review Board (IRB-16-00791; IRB-20-03374). All participants provided informed consent prior to collection of specimen and clinical information. All specimens were coded prior to processing.

Preparation of virus stocks and virus sequencing:

Virus stocks were rescued and grown on Vero E6 cells (multiplicity of infection: 0.05) in infection medium (DMEM+ 2% FBS+ 1% non-essential amino acids) for 3 days. The E484K mutant rSARS-CoV-2 was generated using previously described reverse genetics based on the use of a bacterial artificial chromosome (BAC).[1–3] Viral RNA was extracted from 1ml virus supernatant with TRIzol reagent (ThermoFisher Scientific) according to the manufacturer's instruction. For Sanger sequencing, RT-PCR amplification of the viral genome spanning nucleotides 22853 to 24027 was performed using SuperScript II reverse transcriptase (ThermoFisher Scientific) and the Expand high-fidelity PCR system (Sigma-Aldrich). The 1,175 bp amplified RT-PCR products were subjected to Sanger sequencing (ACGT). The primers used for RT-PCR are available upon request. For deep sequencing of the entire viral genome, we generated libraries using a KAPA RNA HyperPrep kit with a 45-min adapter ligation incubation, including 6 cycles of PCR with 100 ng of viral RNA and a 7 mM adapter concentration. Samples were sequenced on an Illumina HiSeq X machine. The sequencing data were assembled and aligned a SARS-CoV-2 reference genome (GenBank accession no. MN985325) by Integrative Genomic Viewer (IGV 2.9.0).

References

1 Chiem K, Morales Vasquez D, Park J-G, *et al.* Generation and Characterization of recombinant SARS-CoV-2 expressing reporter genes. *J Virol* 2021; published online Jan 11. DOI:10.1128/JVI.02209-20.

2 Chiem K, Ye C, Martinez-Sobrido L. Generation of Recombinant SARS-CoV-2 Using a Bacterial Artificial Chromosome. *Curr Protoc Microbiol* 2020; **59**. DOI:10.1002/cpmc.126.

3 Ye C, Chiem K, Park J-G, *et al.* Rescue of SARS-CoV-2 from a Single Bacterial Artificial Chromosome. *mBio* 2020; **11**. DOI:10.1128/mBio.02168-20.

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The Personalized Virology Initiative study group declares no competing interests.