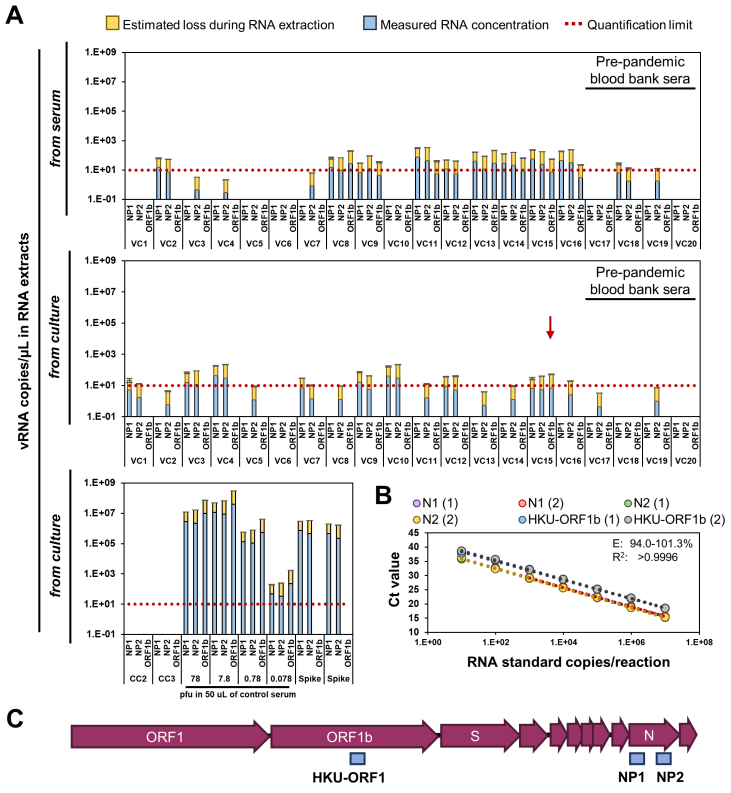
SARS-CoV-2 RNA detected in blood samples from patients with COVID-19 is not associated with infectious virus

Underlying data 2

(i) Quantification of vRNA from serum and culture. (A) Plots show vRNA copy number per µL in RNA extracts from the original VC1 – VC20 serum samples (top panel) and corresponding three day culture samples (middle panel). Lower panel shows positive (spiked N-gene RNA controls and Victoria/01/2020 SARS-CoV-2 passage 4 (Oxford) at calculated ten-fold serial dilutions per well of 78, 7.8, 0.78 and 0.078 pfu in 50 uL of control serum) and negative (CC2 and CC3) control cultures. RNA extracts were analysed using qRT-PCR assays with CDC NP1, CDC NP2 and HKU-ORF1b primer/probe sets, as indicated for each sample. Highlighted in top and middle panel are pre-pandemic blood bank sera (VC17 – VC20). Red arrow points to VC15 (middle panel), as it is the only culture were all three assays gave a low positive signal. Error bars show standard deviation between triplicates. (B) vRNA copy numbers in (A) were interpolated in each qRT-PCR experiment from standard curves of Ct value by known copy number. We provide the efficiency range (E) and smallest R^2 value from all curves. Slope, R^2 and efficiency for each run are as follows: NP1 (1: y=-1.476ln(x)+39.381, R^2 = 0.9999, E=96.9%; 2: y=-1.477ln(x)+39.324, R^2 = 0.9998, E=96.8%), NP2 (1: y=-1.491ln(x)+39.244, R^2 = 1, E=95.6%; 2: y=-1.509ln(x)+39.503, R^2 = 0.9998, E=94.0%) and HKU-ORF1b (1: y=-1.43ln(x)+41.642, R^2 = 0.9987, E=101.3%; 2: y=-1.469ln(x)+42.139, R^2 = 0.9996, E=97.5%). (C) Cartoon highlights genomic location of CDC NP1, CDC NP2 and HKU-ORF1b primer/probe sets in SARS-CoV-2 genome.



(ii) Quantification of vRNA from serum and culture. Table shows vRNA quantification results for analysis of RNA extracts from the original VC1 – VC20 serum samples and corresponding three day culture samples, as well as positive (spiked N-gene RNA controls and Victoria/01/2020 SARS-CoV-2 passage 4 (Oxford) at calculated ten-fold serial dilutions per well of 78, 7.8, 0.78 and 0.078 pfu in 50 uL of control serum) and negative (CC2 and CC3) control cultures. RNA extracts were analysed using qRT-PCR assays with CDC NP1, CDC NP2 and HKU-ORF1b primer/probe sets, as indicated for each sample. Rxn: qRT-PCR reaction set-up; Avg Ct: Average Ct; Cp/µL: Caluculated vRNA copy number per µL in RNA extract; SD: Standard deviation between triplicates for vRNA copy number per µL in RNA extract.

		NP1							NP2							HKU-ORF1b						
Sample		Rxn	Avg Ct	Ct 1	Ct 2	Ct 3	Cp/µL	SD	Rxn	Avg Ct	Ct 1	Ct 2	Ct 3	Cp/µL	SD	Rxn	Avg Ct	Ct 1	Ct 2	Ct 3	Cp/µL	SD
from serum	VC1	20µL reaction with 5µL sample; samples diluted 1 in 10							10µL reaction with 2.5µL sample; samples not diluted							10µL reaction with 2.5µL sample; samples not diluted						
	VC2		36.4	36.3	36.0	36.9	15.0	4.5		35.0	34.8	34.8	35.3	7.0	1.3			38.8				
	VC3									39.1	39.1		39.1	0.4	0.0			38.9				
	VC4					38.6				39.7		39.9	39.6	0.3	0.0			37.5				
	VC5																					
	VC6																					
	VC7				38.4					38.1	38.2	38.2	37.9	0.8	0.1							
	VC8		36.4	36.7	35.4	37.0	15.3	10.3		34.6	34.5	34.6	34.6	9.3	0.3		35.6	35.3	36.0		27.0	9.5
	VC9		37.5		37.6	37.5	7.0	0.2		34.1	34.2	34.2	34.1	12.2	0.7		38.2	37.2	38.7	38.6	4.5	3.4
	VC10																					
	VC11		34.1	34.5	33.8	33.8	73.2	18.9		32.2	32.2	32.1	32.3	44.8	3.3		37.9	36.8	38.6	38.3	5.4	4.6
	VC12		36.8	37.0	36.7	36.7	11.6	1.3		35.3	35.0	35.4	35.5	5.6	0.9							
	VC13		35.0	34.7	35.2	35.1	38.6	6.3		34.2	33.9	34.5	34.4	11.5	2.7		35.6	35.3	35.8	35.6	28.2	4.9
	VC14		35.4	35.3	35.7	35.3	29.2	3.9		33.3	33.4	33.1	33.5	21.3	3.2		37.2	36.6	37.8	37.3	8.7	3.9
	VC15		34.5	34.7	34.1	34.6	55.7	14.1		33.1	33.2	33.1	33.1	24.1	1.3		37.5	37.1	37.5	37.9	7.3	2.3
	VC16		34.8	34.9	35.0	34.5	44.4	9.5		32.7	32.7	32.5	33.0	32.2	5.4		38.8	39.0	38.0	39.4	3.0	1.6
	VC17																					
	VC18		37.7		38.4	37.0	6.3	4.3		37.0	37.9	35.9	37.3	1.8	1.5							
	VC19									37.1		37.1	37.0	1.7	0.0			38.8				
	VC20										39.5											
from culture	VC1		38.0	36.5	39.0	38.5	5.2	6.4		37.0	36.4	37.1	37.7	1.7	0.8							
	VC2				37.6					38.7	39.6	38.3	38.2	0.6	0.3							
	VC3		36.4	37.3	36.1	35.7	15.6	8.2		34.2	34.2	34.2	34.3	11.6	0.3							
	VC4		34.9	35.1	34.5	35.0	42.8	9.9		32.8	32.6	33.2	32.7	29.5	6.2					39.4		
	VC5									37.8	38.8	37.9	36.9	1.2	0.7							
	VC6			38.6																		
	VC7		37.5	37.5		37.4	7.0	0.3		37.6	36.8	36.9	39.3	1.4	1.0							
	VC8			37.3						37.8	37.7	37.7	38.1	1.2	0.2							
	VC9		36.2	35.9	35.6	37.0	16.8	7.5		35.5	35.0	35.5	36.1	5.6	1.7							
	VC10		34.9	35.1	35.3	34.3	40.2	17.0		33.0	33.0	33.2	32.9	29.7	2.5							
	VC11			38.4						37.4	38.4	36.6	37.0	1.7	0.8					38.1		
	VC12		37.1	37.5	37.0	36.9	8.9	1.7		35.6	34.8	36.1	35.9	5.3	2.6							
	VC13									39.1	38.9	39.2		0.5	0.1					38.4		
	VC14			37.6						37.8	38.5		37.1	1.2	0.7							
	VC15		37.6	37.6	38.5	36.6	6.6	4.9		35.6	35.8	35.5	35.4	5.3	0.6		37.9	38.4		37.5	7.0	3.1
	VC16					38.3				36.7	36.0	37.7	36.4	2.6	1.2							
	VC17									39.4	39.2	39.3	39.5	0.4	0.0							
	VC18			38.7																		
	VC19					38.5				38.2		38.3	38.1	1.0	0.1							
	VC20				38.4																	
from culture (controls)	CC2												39.0									
	CC3																					
	78 pfu		18.4	18.4	18.4	18.4	2.9E +06	6.9E +04		16.1	16.1	16.0	16.1	2.2E +06	1.2E +05		17.1	17.2	17.2	17.0	9.9E +06	6.9E +05
	7.8 pfu		16.3	16.4	16.3	16.2	1.2E +07	5.6E +05		13.9	14.0	13.9	13.9	9.2E +06	2.6E +05		15.1	15.1	15.0	15.1	4.0E +07	1.0E +06
	0.78 pfu		22.9	22.9	22.9	22.9	1.4E +05	1.7E +03		20.6	20.6	20.7	20.6	1.1E +05	1.3E +03		21.4	21.5	21.4	21.3	5.4E +05	4.5E +04
	0.078 pfu		34.7	35.0	34.7	34.3	46.8	10.9		32.8	33.0	32.6	32.9	32.9	3.7		32.8	32.8	32.7	32.9	229.3	12.8
	Spike		23.8	23.8	23.8	23.8	7.2E +05	1.2E +04		25.4	25.4	25.5	25.4	4.5E +05	1.4E +04							
	Spike		24.5	24.4	24.5	24.5	4.6E +05	1.1E +04		26.5	26.6	26.3	26.6	2.2E +05	2.8E +04							