

Table S1. Participant characteristics¹ with a confirmed COVID-19 PCR test (M1 to M7). The control group (C1 to C6) represents unexposed mothers who donated human milk samples before the COVID-19 pandemic in 2018.

Mothers	Confirmed COVID-19 PCR (Nasal Swab)	COVID-19 Symptoms	Time from Infection to Collection (Days)	Infant Gender	Lactation Time (Months)	Maternal Age
M1	+COVID19 PCR	Fever, fatigue, cough	61	Female	5	36
M2	+COVID-19 PCR	Cough, fatigue, fever, loss of taste/smell	60	Female	6	26
M3	+RNA SARS-CoV-2 PCR	Fever, nasal congestion, and cough	84	Female	6	26
M4	+COVID-19 PCR	Fatigue, fever	40	Male	6	33
M5	+COVID-19 PCR	Fever and cough	16	Male	8	37
M6	+RNA SARS-CoV-2 PCR	Cough and fever	47	Male	8	31
M7	+COVID-19-PCR	Headaches, fatigue, fever	18	Male	5	33
C1	-	Unknown	-	Male	8	28
C2	-	Unknown	-	Female	5	37
C3	-	Unknown	-	Male	7	29
C4	-	Unknown	-	Female	5	35
C5	-	Unknown	-	Male	5	33
C6	-	Unknown	-	Female	7	27

Women did not have a medical history, medication and systematic diseases.

A S1 subunit SARS-CoV-2

P0DTC2113-685 (YP_009724390.1)
 SQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTFWFAIHVYSG
 TNGTKRFNDPVLFPFNDGVYFASTEKSNIRGWIFGTTLDKSTQSLIVNATNVVVKVCE
 FQFCNDPFLGVYVHKNKSWMESEFRVYSSANCTFEYVQPFMLDLEGKQGNFKNLRE
 VFKNIDGYFKIYKHTPINLVRDLPGQFSALEPLVDLPIGINITRFQTLALHRSYLTGP
 DSSSGWTAGAAAYVGYLQPRFTLLKYNENGTITDAVDCAALDPLSEKCTLKSFTVEKGI
 YQTSNFRVQPTESIVRFPNITLCPFGVEVFNATRFASVYAWNRKRISNCVADYSVLYNSA
 SFSTFKCYGVSPKTLNDLCTNMYADSFVIRGDEVQIAPGGTQKIADYNYLPPDDFTGC
 VIAWNSNLLDSKVGGNVNYLRYLFRKSNLKPFERDISEIYQAGSTPCNGVEGFNCYFPL
 QSYGFPQTPNGVGYQYRVVYVLFELHAPATVCGPKSSTNLVKNKCVNFMNGLTGTGVL
 TESNKKFLPFQGFGRDIADTTDARDPQTELELITPCSFGGVSVITPNTSNQVAGICA
 QDVNCTEVPVAIHADQLTPTWRVYSTGNSVFTQTRAGCLIGAEHVNSYECDDIPGAGICA
 SYQTQNTSPRRAR

C S1+S2 HCoV-229E

A0A1L7B942 (APT69883.1)
 MFVLLVAYALLHIAGCQTNGTNTSHSVCNGCVGHSNENFAVESGGYIPSNFNFNNWFL
 TNSVVDGVVRSFQPLLNLCLVSVSGSOFTTGFVYFNGTGRGACKGFYSNASSDVIRYN
 IFEENLRRTILFKTSYGAVVFCYNTNLTVSGDAHIPSGTVLGNFYCFVNTTIGNETTS
 AFVGLPKTREFVISRTHGFHYNGYRYSFLGDVEAVNFVNATNATVCTVALASVADVL
 VNSQTAIANIYCNVSNIRLRCDDLSDFPDGCFYSTSIQIPVLEPMSIVSLPVYHKHTF
 IULVHKFEHQRGPKCYNCRPSVINILANFNETKGPLCVDTSHTFTQVDFVNDKLRWISA
 SINTGNCPPFSFGKVNFKVGSVFLSKDIPGGCAMPIMANLVNHNKSHNIGSLYVYSWDG
 DVTITGVPPKVEGVSSFMNVTLNKCTKYNIYDVSQVGVIRISNDTFLNGITYTSTSNLLG
 FKDVNTGTYITPCNPPDQLVYVQQAVVGAMLENFTSYGFSNVEMPKFFYASNGTYN
 CTDAVLTYSFSGVQCADGSIIVQPRNVSYDSVAIVTANLSIPSNTWTSVQVEYLQITSK
 PIVVDCSTYVCGNVRCELLKQYTSACKTIEDALRNSAMLESADVSEMLTFDKKAFTLA
 NVSSFGDYNLSSVPSLPRSGSRVAGRSIAIEDILFSLKLVTSGLGTVDADYKCKTKGLSIA
 DLACAQYNGIMVLPGVADAERMAMYTGSLIGGIALGGLTSAASIPFSLAISQRLNVAL
 QTDVLEQENKILAAASFNKAMTINIVDAFTGVNDAITQTSQALQTVATALNKIQDVVNQGN
 SLNHLTSQLRQNFQAISSIIQIYDRLDIIQQDQVDRITGRALALNVFVSHLTLTYTE
 VRASRQLAQKQVNECVKSSQSRKGYFCGNGTHIFSLVNAEPLVFLHTVLLPTQYKDVEA
 VWSGLCVDGNGVYLRQPNLALYKEGNYRITSRIMFEPRIPTIADVQIENCNVTVFNIS
 RSELQTVIPEYIDVNLQELSYKLPNYTVPDLVVEQYNQTLNLTSEISTLENKSAELN
 YTVQKQLTLIDININSLVDLKLWLNRYETIKWPWWWLCLISVVLIFVWSMLLCCCSTGC
 CGFFSCFASIKGCCSTKLPYVYDEKIHQ

B S2 subunit SARS-CoV-2

P0DTC21686-1273 (YP_009724390.1)
 SVASQSIAYTMSLGAENSVAYSNNSIAIPTNFTISVTEILPVSMTKTSVDCTMYICGD
 STECSNLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQI
 LPDPSKPSKRSFIEDLLFNKVTADAGFIKQYDGLDIAARDLCAQKFNGLTVLPLLL
 TDEMAIQTALLAGTITSGWTFGAGAAALQIPFAMQMAYRFRNGIVTQNVLYENQKLIAN
 QFNSAIGKIDLSSTASALGKLDQVNVNQAQALNLTIVLQSSNFGAISVLDLISRLD
 KYEAQVQIDRLITGRQLSLQTYVTOQLIRAAEIRASANLAATKMSCEVLGQSKRVDFCGK
 GYHLSMFPQSAPHGVVFLHVTYVPAQEKNTFTAPACHDGAHFREGVFNSTGTHWFTV
 QRNFYEQIITDNTFVSGNCDVIGIVNNTVYDPLQPELDSFKEELDKYKNIHSTPVDV
 LGDISGINASVNIQKIDRLNEVAKNLNESLIDQLQELGKVEYQIKWPPYIWLFIAGLI
 AIVMVTMLCCMTCSCCCKGKCCSCGSCCKFDEDDSEPVKLVKGLHY

D S1+S2 HCoV-OC43

A0A2P1ZWW8 (AVR40344.1)
 MFLILLISLPTAFVIGDLNCTLDPRKGSFNRRDTPGSSISIDTVDVNTGLGTYVYVLDL
 VYLNLTFLNGYPTSGSTYRNMALKGTDLSTLWFKPPFLSDFINGIFAKVNTKVKFD
 GVMYSEFPAITIGTFVNTSYSVVQPRNTINSQDGVNKLQGLLEVSVCQYNMCEYPHTI
 CHPNLGNHFKELWHYDTGVVSVCLYKRNFTYDVAATLYVHFYQEGGTFYAYFTDGFVTK
 FLNFVYLGMLSHYVMPLTGIRRPKDGFSLEYVWVTPRQYLLAFNQDGIIFNAVDCM
 SDFMSEIKCKTQSIAPPTGVYELNGYTVQVADVYRKRDPDLPCNIEAWLNDKSVSPLN
 WERKTFSNCFNMSLSMFIQADSFTCNNDIAAKIYGMCFSSITIDKFAIPNRRKVDLQ
 GNGLVQSSNIRIDTATSCQLYNNLPAANVSVSRFPSTWNRKRFIEDSVFVQPTGV
 FTNHSVYVYQHCFAKPKNFCPCSSCPCGKNGIGTAPAGTNSLTDNLCTLDPITLKAP
 DTYKCPQSKLVGIGEHCSGLAVKSDYCGNNSCTQOPQALGWSADSCQGDCKNIFANF
 ILHDVNNGLTCDTDLQKANTEIELGVCVNYDLYGISGGIFVEVNTATYNSWONLLYDSN
 GNLYGFRDITNRTFMHSCYSGRVSAAYHANSSEPALFRNIKCNVFNNSLTRLQLOPI
 NYSFDSYLCVVNAYNSTAISVGTCDLTVGSGYCVDYSKNRRSRAITTYRFTNFEFPT
 VNSVNDSELPVGGLEYIQIPSEFTIGNMEEFIQTSSPKVITDCAAFVCGDYAACKLQVE
 YGSCFDNINAILTEVNEILLDTTQLQVANSLMNGVTLSTKLKDVGNVFNVDINFPVLGCL
 GSECSKASSRSIAEDLFDKVKLSDVGFVEAYNNTGGAEIRDLCVQSYKIGKVLPLLL
 SENQISGYTLAATSASLFPWTAAGVFPYLVNVRINGLGVMTDVLQSQKLIANAFNN
 ALHAIQGGFDATNSALVKIQAVNANAEALNLLQQLSNRFGAISASLQELISRLDALEA
 EAQIDRLINGRLTALNAYVSQLSDSTLVKFAAQAMEKNEVCVKSQSSRNFCGNGNHI
 ISLVQNPAPYGLYFIHFNYPTKYVYAKVSPGLCIAGNRGIAPKSGYFVNVNNTWMTYGS
 YYYPEPITENNVMVMSSTCAVNYTKAPVYMLNTPDFKEELDQWFKNQTSVAPDLV
 DYINVTLDLQVEMNRLQEAIKVLNHSYINLKDITGYEYVWVWVWVWVWVWVWVWVWVWV
 LLFFICCTGCGTSCFKKCGGCCDDYTYGQELVKTSHDD

Figure S1. Amino acid sequences from (A) S1 subunit and (B) S2 subunit from SARS-CoV-2, S1 + S2 subunits from (C) HCoV-229E and (D) HCoV-OC43. Sequences were from UniProt (ELIXIR core data resource).

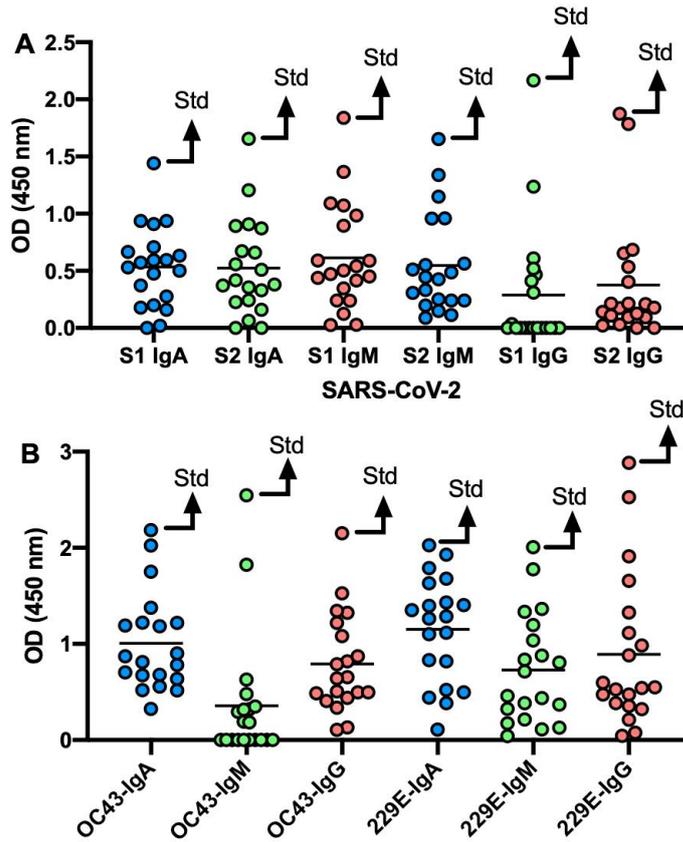


Figure S2. Selection of standards (std) with the highest optical density (OD) values using ELISA. The OD values for antibody secretory IgA (SIgA)/IgA, secretory IgM (SIgM)/IgM, and IgG reactive to (A) S1 or S2 subunit SARS-CoV-2, (B) S1+S2 subunits HCoV-OC43, or S1+S2 subunits HCoV-229E. The standards were selected among 20 human milk samples (supernatants) diluted at 10x for IgG and IgM and at 25x for SIgA/IgA in blocking buffer.

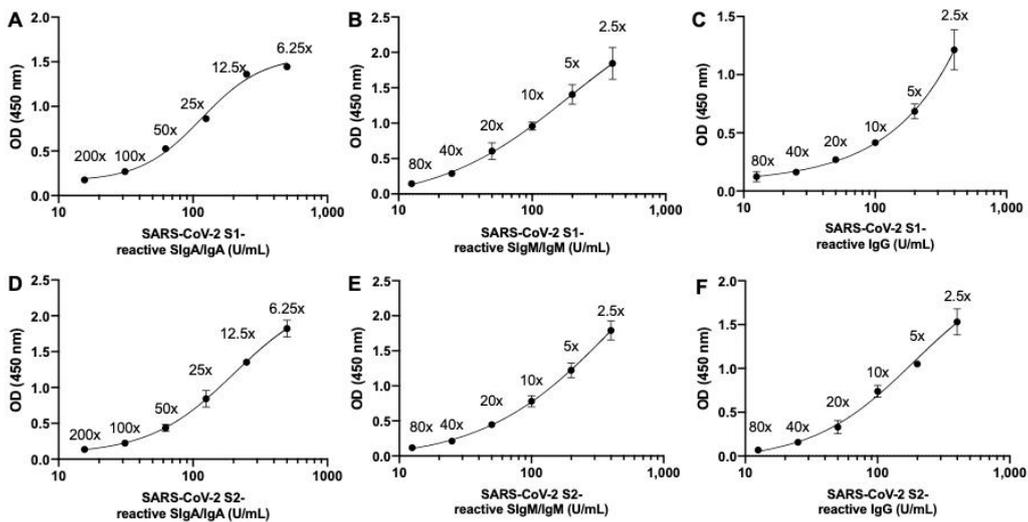


Figure S3. Standard curves used to determine the levels of antibodies reactive to SARS-CoV-2 S1 or S2 subunit. Standard curves for SARS-CoV-2 S1-reactive **a** secretory IgA (SIgA)/IgA, **b** secretory IgM (SIgM)/IgM, and **c** IgG. Standard curves for SARS-CoV-2 S2-reactive **d** SIgM/IgM, **e** SIgA/IgA, and **f** IgG. Values are means \pm SD, $n = 3$ for experiments. The standard curves were prepared using the human milk supernatant with the highest optical density values (see Figure S2).

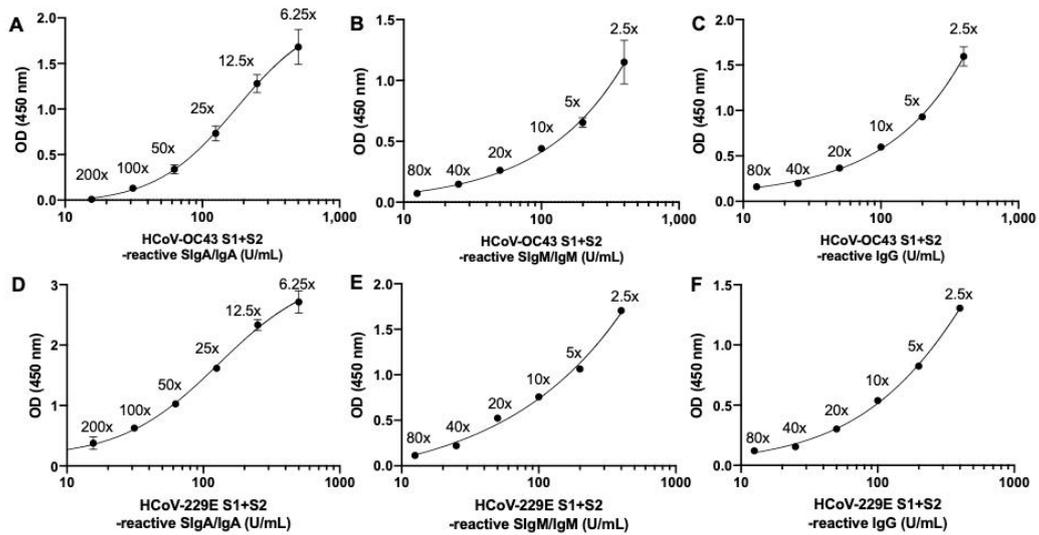


Figure S4. Standard curves used to determine the levels of antibodies reactive to HCoV-OC43 and HCoV-229E S1+S2 subunits. Standard curves for HCoV-OC43 S1+S2-reactive **a** secretory IgA (SIgA)/IgA, **b** secretory IgM (SIgM)/IgM, and **c** IgG. Values are means \pm SD, $n = 3$ for experiments. The standards were prepared using the human milk supernatant with the highest optical density values (see Figure S2).