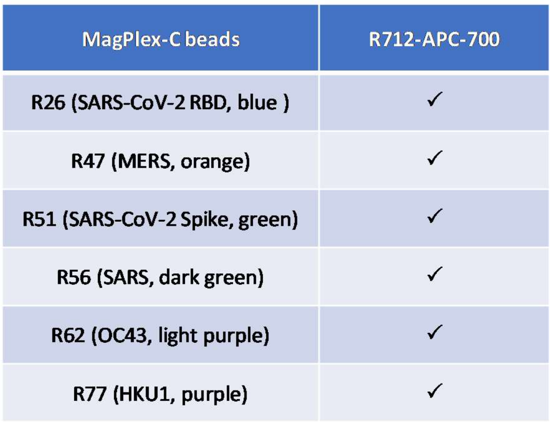
Supplemental Information

Figure S1. Each viral antigen listed in the table was coupled to a microbead population with a distinct address, e.g., R26 for SARS-CoV-2 RBD that displayed a different fluorescence intensity in the fluorescence channel, R712-APC-700 of the CytoFLEX cytometer (right plot). Because the serology assays and assays assessing cross reactivity were performed separately in different sample wells, assay performance was not affected by the overlap of the intensity addresses of R51 (spike) and R56 (SARS).



MagPlex-C beads	R712-APC-700
R26 (SARS-CoV-2 RBD, blue)	✓
R47 (MERS, orange)	✓
R51 (SARS-CoV-2 Spike, green)	✓
R56 (SARS, dark green)	✓
R62 (OC43, light purple)	✓
R77 (HKU1, purple)	✓

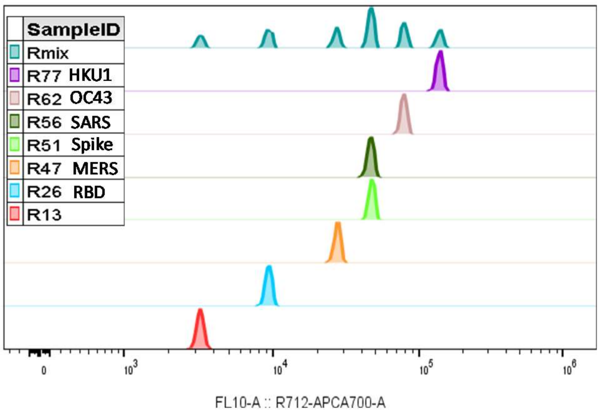
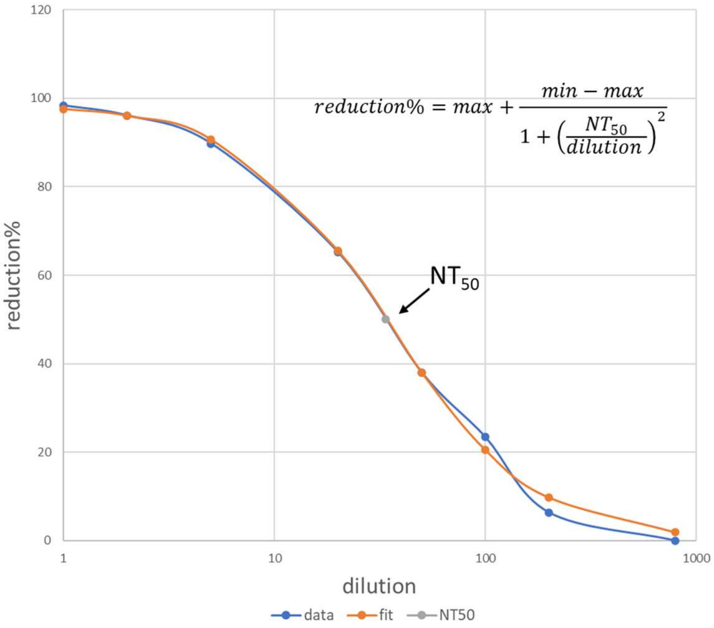


Figure S2. A plot of sample dilution vs. reduction % is shown for a positive convalescent patient sample as an example. The NT_{50} (grey dot) was determined by modeling the does-response curve shown as red line using Eq. 4 provided in the figure.



Supplemental coronaviruses spike protein sequences: Comparison of the full length spike protein sequence for R619-X16: SARS-CoV2 S-2P-3C-His8-Strep2x2 (SARS-COV2), R619-X17: SARS-CoV S-2P-3C-His8-Strep2 (SARS-CoV), R619-X18: MERS-CoV S-2P-3C-His8-Strep2x2 (MERS-CoV), R619-X19: OC43 S-2P-3C-His8-Strep2x2 (OC43), and R619-X20: HKU1 S-2P-3C-His8-Strep2 (HKU1). The S1 section is in blue text, the RBD section green text and the S2 section purple text. The highlights indicate the homology compared with SARS-Cov2. Blue indicates all five sequences have the same the amino acid, pink indicates four of the five sequences, green for three and yellow for two sequences the same. Abbreviations: 3C, rhinovirus 3C protease cleavage site; Strep2x2, dual Strep2 epitope tag; T7, bacteriophage T7 fibritin trimerization domain; SBP, streptavidin binding peptide. The sequence alignment was carried out using Clustal Omega program (https://www.ebi.ac.uk/Tools/msa/clustalo/).

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                                   S1
SARS-CoV2       ------------CVNLT---------TRTQL---PPAYTNSFTRGVYYPDKVFRSSVLHS 36
SARS-CoV        -------SDLDRCTTFD---------DVQAP---NYTQHTSSMRGVYYPDEIFRSDTLYL 41
MERS-CoV        YVDVGPDSVKSACIEVDIQQT---FFDKTWP---R-PIDVSKADGIIYPQGRTYSNITIT 53
OC43            --VIG----DLKCPL-DSRTGSLNNIDTGPPSISTATVDVTNGLGTYYVLDRVYLNTTLF 53
HKU1            --VIG----DFNCTN-----SFINDYNKTIPRISEDVVDVSLGLGTYYVLNRVYLNTTLL 49

SARS-CoV2       TQDLFLPFFSNVT---WFHAIHVSGTNGTKR----FDNP-VLPFNDGVYFAST------- 81
SARS-CoV        TQDLFLPFYSNVT---GFHTIN-------HT----FGNP-VIPFKDGIYFAAT------- 79
MERS-CoV        YQGLF-PYQGDHGDMYVYSAGHATGTTPQKLFVANYSQD-VKQFANGFVVRIGAAANSTG 111
OC43            LNGYYPTSGSTYRNMA------LKGTDKLS--TLWFKPPFLSDFINGIFAKVKNTKVFKD 105
HKU1            FTGYFPKSGANFRDLA------LKGSIYLS--TLWYKPPFLSDFNNGIFSKVKNTKLYVN 101

SARS-CoV2       ------E----KSNIIRGWIFGTTLDSKTQ---------SLLIVNN-------A-----T 110
SARS-CoV        ------E----KSNVVRGWVFGSTMNNKSQ---------SVIIINN-------S-----T 108
MERS-CoV        TVIISPSTSATIRKIYPAFMLGSSVGNFSDGKMGRFFNHTLVLLPD-------G-----C 159
OC43            GV---------MYSEFPAITIGSTFVNTSY---------SVVVQPRTINSTQDGVNKLQG 147
HKU1            NT---------LYSEFSTIVIGSVFVNTSY---------TIVVQPH------------NG 131

SARS-CoV2       NVVIKVCEFQFCNDPFLGVYYHKN---------------------NKSWMESEFRVYSSA 149
SARS-CoV        NVVIRACNFELCDNPFFAVSKPMG---------------------T----QTHTMIFDNA 143
MERS-CoV        GTLLRAF--YCILEPRSGNHCPAGNSYTSFATYHTPATDCSDGNYNRNASLNSFKEYFNL 217
OC43            LLEVSVCQYNMCEYPHTICHPKLGNHF------------------KELWHMDTGV-VSCL 188
HKU1            ILEITACQYTMCEYPHTVCKSK-GSIR------------------NESWHIDSSE-PLCL 171

SARS-CoV2       NNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSAL 209
SARS-CoV        FNCTFEYISDAFSLDVSEKSGNFKHLREFVFKNKDGFLYVYKGYQPIDVVRDLPSGFNTL 203
MERS-CoV        RNCTFMYTYNITEDEILEWFGITQTAQG-VHLFSSRYVDLYGGN---------------M 261
OC43            YKRNFTYDVNATYL-------------YFHFYQEGGTFYAYFTDT--GVV---------T 224
HKU1            FKKNFTYNVSADWL-------------YFHFYQERGVFYAYYADV--GMP---------T 207

SARS-CoV2       EPLVDLPIGINITRFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENG 269
SARS-CoV        KPIFKLPLGINITNFRAILTAFS------PAQDIWGTSAAAYFVGYLKPTTFMLKYDENG 257
MERS-CoV        FQFATLPVYDTIKYYSIIPHSIR---SIQSDRKAW----AAFYVYKLQPLTFLLDFSVDG 314
OC43            KFLFNVYLGMALSHYYVMPLTCI---S--R---RDIGFTLEYWVTPLTSRQYLLAFNQDG 276
HKU1            TFLFSLYLGTILSHYYVMPLTCN---AISS---NTDNETLEYWVTPLSRRQYLLNFDEHG 261

                                                       RBD
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SARS-CoV2     TITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFR VQPTESIVRFPNITNLCPFGEVFN 329
SARS-CoV      TITDAVDCSQNPLAELKCSVKSFEIDKGIYQTSNFR VVPSGDVVRFPNITNLCPFGEVFN 317
MERS-CoV      YIRRAIDCGFNDLSQLHCSYESFDVESGVYSVSSFE AKPSGSVVEQAE-GVECDFSPLLS 373
OC43          IIFNAVDCMSDFMSEIKCKTQSIAPPTGVYELNGYT VQPIADVYRRKPDLPNCNIEAWLN 336
HKU1          VITNAVDCSSSFLSEIQCKTQSFAPNTGVYDLSGFT VKPVATVYRRIPNLPDCDIDNWLN 321

SARS-CoV2     ATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIR 389
SARS-CoV      ATKFPSVYAWERKKISNCVADYSVLYNSTFFSTFKCYGVSATKLNDLCFSNVYADSFVVK 377
MERS-CoV      G-TPPQVYNFKRLVFTNCNYNLTKLLSLFSVNDFTCSQISPAAIASNCYSSLILDYFSYP 432
OC43          DKSVPSPLNWERKTFSNCNFNMSSLMSFIQADSFTCNNIDAAKIYGMCFSSITIDKFAIP 396
HKU1          NVSVPSPLNWERRIFSNCNFNLSTLLRLVHVDSFSCNNLDKSKIFGSCFNSITVDKFAIP 381

SARS-CoV2     GDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNL-- 447
SARS-CoV      GDDVRQIAPGQTGVIADYNYKLPDDFMGCVLAWNTRNIDATSTGNYNYKYRYLRHGKL-- 435
MERS-CoV      LSMKSDLSVSSAGPISQFNYKQSFSNPTCLILATVPHNLTTITKPLKYSYINKCSRFL-- 490
OC43          NGRKVDLQLGNLGYLQSFNYRIDTTATSCQLYYNLPAANVSVSR-FNPSTWNKRFGFIEN 455
HKU1          NRRRDDLQLGSSGFLQSSNYKIDISSSSCQLYYSLPLVNVTINN-FNPSSWNRRYGFGSF 440

SARS-CoV2     ---KPFER--------DISTEIYQ--AGSTPCNGV-----------------EG-----F 472
SARS-CoV      ---RPFER--------DISNVPFS--PDGKPCTP------------------PA-----L 459
MERS-CoV      ---SDDRT--------EVPQLVNA--NQYSPCVSI-----------------VP-----S 515
OC43          SVFKPQPAGVLTNHDVVYAQHCFKAPKNFCPCKLNSSLCVGSGPGKNNGIGTCPAGTNYL 515
HKU1          N---------LSSYDVVYSDHCFSVNSDFCPCADPSVVN--SCAKSKPPSAICPAGTKYR 489


SARS-CoV2     NCYF-----------------PLQSYGFQ-----------------------------PT 486
SARS-CoV      NCYW-----------------PLNDYGFY-----------------------------TT 473
MERS-CoV      T-VWEDGDYYRK------QLSPLEGGGWL-----------------------------VA 539
OC43          TC------------HNLCNPDPITFTGPYKCPQTKSLVGIGEHCSGLAVKSDYCGG---- 559
HKU1          HCDLDTTLYVKNWCRCSCLPDPISTYSPNTCPQKKVVVGIGEHCPGLGINEEKCGTQLNH 549

SARS-CoV2     NGVGYQPYRVVVLSFEL------------L----HAPATVCGP-----KKSTNLVKNKCV 525
SARS-CoV      TGIGYQPYRVVVLSFEL------------L----NAPATVCGP-----KLSTDLIKNQCV 512
MERS-CoV      SGSTVAMTEQLQMGFGI------------TVQYGTDTNSVCPKLEFANDTKIASQLGNCV 587
OC43          NPCTCQPQAFLGWSADSCLQGDKCNIFANLILHDVNSGLTCSTD--LQKANTDIKLGVCV 617
HKU1          SSCFCSPDAFLGWSFDSCISNNRCNIFSNFIFNGINSGTTCSND--LLYSNTEISTGVCV 607

             End of RBD
SARS-CoV2    NF NFNGLTGTGVLTESNKKF-LPFQQFGRDIADTT-DAVRDPQTLEILDITPCSFGGVSV 583
SARS-CoV     NF NFNGLTGTGVLTPSSKRF-QPFQQFGRDVSDFT-DSVRDPKTSEILDISPCAFGGVSV 570
MERS-CoV     EY SLYGVSGRGVFQNCTAVG-VRQQRFVYDAYQNLVGYYSD--DGNYYCLRACVSVPVSV 644
OC43         NY DLYGISGQGIFVEVNATYYNSWQNLLYDSNGNLY-GFRDYITNRTFMIRSCYSGRVSA 676
HKU1         NY DLYGITGQGIFKEVSAAYYNNWQNLLYDSNGNII-GFKDFLTNKTYTILPCYSGRVSA 666

SARS-CoV2     ITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLT--PTWRVYSTGSNVFQTRAGCLIGAEH 641
SARS-CoV      ITPGTNASSEVAVLYQDVNCTDVSTAIHADQLT--PAWRIYSTGNNVFQTQAGCLIGAEH 628
MERS-CoV      IYD--KETKTHATLFGSVACEHISSTMSQYSRSTRSMLKRRDSTYGPLQTPVGCVLGLVN 702
OC43          AFH--ANSSEPALLFRNIKCNYVFNNSLIRQLQ----------PINYFDSYLGCVVNAYN 724
HKU1          AFY--QNSSSPALLYRNLKCSYVLNNIS--FIS----------QPFYFDSYLGCVLNAVN 712

                                                beginning of S2
SARS-CoV2     VN--NSYECDIPIGAGICASYQTQTNSP-GSAS SVAS---QS-IIAYTMSLGA-ENSVAY 693
SARS-CoV      VD--TSYECDIPIGAGICASYHTVSLL-----R STSQ---KS-IVAYTMSLGA-DSSIAY 676
MERS-CoV      SS-LFVEDCKLPLGQSLCALPDTPSTLTPASVG SVPGEMRLA-SIAFNHPIQV--DQLNS 758
OC43          STAISVQTCDLTVGSGYCVDYSKNR--RSRRAI TTGY--RFTNFEPFTVNSVNDSLEPVG 780
HKU1          LTSYSVSSCDLRMGSGFCIDYALPSSGGSGSGI SSPY--RFVTFEPFNVSFVNDSVETVG 770


SARS-CoV2     SNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRAL 753
SARS-CoV      SNNTIAIPTNFSISITTEVMPVSMAKTSVDCNMYICGDSTECANLLLQYGSFCTQLNRAL 736
MERS-CoV      SYFKLSIPTNFSFGVTQEYIQTTIQKVTVDCKQYVCNGFQKCEQLLREYGQFCSKINQAL 818
OC43          GLYEIQIPSEFTIGNMEEFIQTSSPKVTIDCAAFVCGDYAACKSQLVEYGSFCDNINAIL 840
HKU1          GLFEIQIPTNFTIAGHEEFIQTSSPKVTIDCSAFVCSNYAACHDLLSEYGTFCDNINSIL 830
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SARS-CoV2      TGIAVEQDKNTQEVFAQVKQIYKTPP-----IKDFGGF-NFSQILP-DP---SKPSKRSF 803
SARS-CoV       SGIAAEQDRNTREVFAQVKQMYKTPT-----LKYFGGF-NFSQILP-DP---LKPTKRSF 786
MERS-CoV       HGANLRQDDSVRNLFASVKSSQSSPI-----IPGFGGDFNLTLLEP-VSISTGSRSARSA 872
OC43           TEVNELLDTTQLQVANSLMNGVTLSTKLKDGVNFNVDDINFSSVLGCLGSECSKASSRSA 900
HKU1           NEVNDLLDITQLQVANALMQGVTLSSNLNTNLHSDVDNIDFKSLLGCLGSQCGS-SSRSL 889

SARS-CoV2      IEDLLFNKVTLADAGFIKQYGDCL--GDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTS 861
SARS-CoV       IEDLLFNKVTLADAGFMKQYGECL--GDINARDLICAQKFNGLTVLPPLLTDDMIAAYTA 844
MERS-CoV       IEDLLFDKVTIADPGYMQGYDDCMQQGPASARDLICAQYVAGYKVLPPLMDVNMEAAYTS 932
OC43           IEDLLFDKVKLSDVGFVAAYNNCT--GGAEIRDLICVQSYKGIKVLPPLLSENQISGYTL 958
HKU1           LEDLLFNKVKLSDVGFVEAYNNCT--GGSEIRDLLCVQSFNGIKVLPPILSETQISGYTT 947

SARS-CoV2      ALLAGTITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQ 921
SARS-CoV       ALVSGTATAGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKQIANQFNKAISQIQ 904
MERS-CoV       SLLGSIAGVGWTAGLSSFAAIPFAQSIFYRLNGVGITQQVLSENQKLIANKFNQALGAMQ 992
OC43           AATSASLFPPWTAA----AGVPFYLNVQYRINGLGVTMDVLSQNQKLIANAFNNALDAIQ 1014
HKU1           AATVAAMFPPWSAA----AGVPFSLNVQYRINGLGVTMDVLNKNQKLIANAFNKALLSIQ 1003

SARS-CoV2      DSLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDPPEAEVQIDR 981
SARS-CoV       ESLTTTSTALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDPPEAEVQIDR 964
MERS-CoV       TGFTTTNEAFHKVQDAVNNNAQALSKLASELSNTFGAISASIGDIIQRLDPPEQDAQIDR 1052
OC43           EGFDATNSALVKIQAVVNANAEALNNLLQQLSNRFGAISSSLQEILSRLDPPEAEAQIDR 1074
HKU1           NGFTATNSALAKIQSVVNANAQALNSLLQQLFNKFGAISSSLQEILSRLDPPEAQVQIDR 1063


SARS-CoV2      LITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQS 1041
SARS-CoV       LITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQA 1024
MERS-CoV       LINGRLTTLNAFVAQQLVRSESAALSAQLAKDKVNECVKAQSKRSGFCGQGTHIVSFVVN 1112
OC43           LINGRLTALNAYVSQQLSDSTLVKFSAAQAMEKVNECVKSQSSRINFCGNGNHIISLVQN 1134
HKU1           LINGRLTALNAYVSQQLSDITLIKAGASRAIEKVNECVKSQSPRINFCGNGNHILSLVQN 1123

SARS-CoV2      APHGVVFLHVTYVPAQEKNFTTAPAICHDGKA---HFPREGVFVSN-----GTHWFVTQR 1093
SARS-CoV       APHGVVFLHVTYVPSQERNFTTAPAICHEGKA---YFPREGVFVFN-----GTSWFITQR 1076
MERS-CoV       APNGLYFMHVGYYPSNHIEVVSAYGLCDAANPTNCIAPVNGYFIKTNNTRIVDEWSYTGS 1172
OC43           APYGLYFIHFSYVPTKYVTAKVSPGLCIAGDR--GIAPKSGYFVNV-----NNTWMYTGS 1187
HKU1           APYGLLFIHFSYKPTSFKTVLVSPGLCLSGDR--GIAPKQGYFIKQ-----NDSWMFTGS 1176

SARS-CoV2      NFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQ--PELDSFKEELDKYFKNHTSPDVD 1151
SARS-CoV       NFFSPQIITTDNTFVSGNCDVVIGIINNTVYDPLQ--PELDSFKEELDKYFKNHTSPDVD 1134
MERS-CoV       SFYAPEPITSLNTKYVAPQVTYQN-ISTNLPPPLLGNSTGIDFQDELDEFFKNVSTSIPN 1231
OC43           GYYYPEPITENNVVVMSTCAVNYTKAPYVML--NTSTPNLPDFREELDQWFKNQTSVAPD 1245
HKU1           SYYYPEPISDKNVVFMNSCSVNFTKAPFIYL--NNSIPNLSDFEAELSLWFKNHTSIAPN 1234

SARS-CoV2      LGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQGSGYIPEAPRDGQAYVR 1211
SARS-CoV       LGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQGSGYIPEAPRDGQAYVR 1194
MERS-CoV       FGSLTQINTTLLDLTYEMLSLQQVVKALNESYIDLKELGNYTYGSGYIPEAPRDGQAYVR 1291
OC43           LSLD-YINVTFLDLQVEMNRLQEAIKVLN--------------GSGYIPEAPRDGQAYVR 1290
HKU1           LTFNSHINATFLDLYYEMNVIQESIKSLN--------------GSGYIPEAPRDGQAYVR 1280

SARS-CoV2      KDGEWVLLSTFLGRSLEVLFQGPGHHHHHHHHSAWSHPQFEKGGGSGGGGSGGSAWSHPQ 1271
SARS-CoV       KDGEWVLLSTFLGRSLEVLFQGPGHHHHHHHHSAWSHPQFEK------------------ 1236
MERS-CoV       KDGEWVLLSTFLGRSLEVLFQGPGHHHHHHHHSAWSHPQFEKGGGSGGGGSGGSAWSHPQ 1351
OC43           KDGEWVLLSTFLGRSLEVLFQGPGHHHHHHHHSAWSHPQFEKGGGSGGGGSGGSAWSHPQ 1350
HKU1           KDGEWVLLSTFLGRSLEVLFQGPGHHHHHHHHSAWSHPQFEK------------------ 1322

SARS-CoV2      FEK       1274
SARS-CoV       ---       1236
MERS-CoV       FEK       1354
OC43           FEK       1353
HKU1           ---       1322
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