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Abstract

The global COVID-19 pandemic has created an urgent demand for accurate rapid point of care diagnostic tests. Antigen-based assays are suitably inexpensive and can be rapidly mass-produced, but sufficiently accurate performance requires highly optimized antibodies and assay conditions. An automated liquid handling system, customized to handle lateral flow immunoassay (LFA) arrays, was used for high-throughput antibody screening of anti-nucleocapsid antibodies that will perform optimally on an LFA. Six hundred seventy-three anti-nucleocapsid antibody pairs were tested as both capture and detection reagents with the goal of finding those pairs that have the greatest affinity for unique epitopes of the nucleocapsid protein of SARS-CoV-2 while also performing optimally in an LFA format. In contrast to traditional antibody screening methods (e.g. ELISA, bio-layer interferometry), the methods described here integrate real-time LFA reaction kinetics and binding directly on nitrocellulose. We have identified several candidate antibody pairs that are suitable for further development of an LFA for SARS-CoV-2.

Introduction

The emergence of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to a global pandemic of COVID-19, infecting more than fifteen million people worldwide in less than 8 months, and killing over 600,000 persons as of late July, 2020.[1,2] Strategies to suppress transmission of SARS-CoV-2, the virus that causes COVID-19, have been constrained by limitations in the availability of tests that can detect viral infection early. The predominant test format used to detect SARS-CoV-2 is reverse transcriptase polymerase chain reaction (RT-PCR), conducted most commonly on specimens collected from the nasopharynx or oropharynx of symptomatic or exposed individuals. Demand for RT-PCR testing for SARS-CoV-2 has in most places exceeded available supply.

Diagnostic testing is central to detecting the virus in persons presenting with and without COVID-19 symptoms, or those identified as contacts exposed to COVID-19 cases, to guide community interventions that are predicted to contain ongoing transmission. The pandemic has resulted in unprecedented demand on the RT-PCR testing capacity of all countries. Demand for testing has been coupled with a global shortage of commercial kits, reagents, consumables, disruptions in the global transport networks, and exacerbated by international competition for testing resources. Accordingly, even many high-income countries have inadequate RT-PCR testing capacity to effectively suppress ongoing transmission, and most low and middle-income countries (LMICs) are unlikely to be able to establish even minimally needed RT-PCR capacity in the immediate future.

Direct antigen-based tests for SARS-CoV-2 offer an attractive alternative solution to testing needs and possibly the only viable solution for most LMICs. Antigen tests, which detect the presence of viral proteins, can be directly

conducted on biological samples, such as tissue swabbed from the anterior nasal cavity, oropharynx, or even directly on saliva. Such antigen tests already exist for influenza, strep throat, and other infectious diseases. LFA antigen tests in particular already have extremely high production capacities in the billions of units/year, are relatively inexpensive and easy to use, return results in minutes, and crucially, like RT-PCR and unlike serological tests, can reveal an active infection.

The use case for a low-cost, highly accessible SARS-CoV-2 assay is strong even if the assay were to be less sensitive than current RT-PCR testing. Modeling shows that decentralized, point-of-care testing with rapid return of results would have substantially greater potential impact on transmission than the absolute limit of detection of the assay.[3] These models build on the important observation that infectious viral particles have not been recovered below around 100 copies/mL.[4,5]

Rapid antigen tests are beginning to enter the commercial market. Thus far, however, few antigen tests for SARS-CoV-2 have received authorization from regulatory authorities worldwide. As of July 19th, 2020, two such products have received emergency use authorization (EUA) from the US Food and Drug Administration.[6,7]

These FDA EUA authorized assays require instrumentation and are not available at low cost or outside health care settings. A concerted effort is underway to catalyze development of antigen-based rapid diagnostic tests that require no or minimal instrumentation, and to prepare manufacturing capability to meet the needs of the larger global market.[8] The required performance characteristics of a SARS-CoV-2 antigen detection assay have not yet been published by the World Health Organization or other entities, but the FIND-UNITAID expression of interest proposes a minimum clinical sensitivity of 80%, and clinical specificity of 97% (compared to RT-PCR), to allow for large scale testing of moderate-risk populations.

A key step in the development of an LFA is the selection of the best antibodies. Our group has pioneered a high-throughput robotic antibody screening process directly on nitrocellulose.[9] This method allows us to rapidly screen hundreds of combinations of antibodies far more quickly than is typical of early-stage LFA development while simultaneously utilizing nitrocellulose-specific reaction kinetics and flow rates that are difficult-to-impossible to mimic in other traditional multiplexed systems (e.g. ELISA, biolayer interferometry). Chemical gradients, residence times, binding orientations, affinity rates, drying and subsequent rehydration of reagents, and spatial distributions of antibodies are different in LFAs than in other immunoassays, and therefore, the best antibodies for LFAs may be different than for the best antibodies for ELISA, for example.

In this paper we describe the results of an extensive antibody screening effort that utilized our high-throughput robotic antibody screening platform[9] to screen through 673 combinations of antibody pairs that target the SARS-CoV-2 nucleocapsid protein.

Materials and Methods

Reagents and materials
The following LFA reagents were purchased: TritonX-100, Tween-20, 10X PBS, sucrose, and IGEPAL (CA-630) from Sigma Aldrich (St. Louis, MO, USA); Surfactant-10G from Fitzgerald Industries (Acton, MA, USA); 20× Borate, pH 8.5 and 10× PBST from Thermo Fisher Scientific (Waltham, MA, USA); PBS tablets from VWR (Radnor, PA, USA); BSA from Seracare Life Sciences (Milford, MA, USA).

SARS-CoV-2 nucleocapsid antigens were purchased from Acro Biosystems (Cat. No. NUN-C5227), Creative Diagnostics (Cat. No. DAGC094), Genemedi (Cat. No. GMP-V-2019nCoV-N002), Genscript (Cat. No. Z03480-1), MyBiosource (Cat. No. MBS7135899), Sino Biological (Cat. No. 40588-V088), and The Native Antigen Co. (Cat. No. REC31812-100). A list of anti-nucleocapsid antibodies screened in this work are provided in Table 2si (supp. info).

The following LFA materials were used for antibody screening: backed nitrocellulose (20 mm wide, CN95, Sartorius Lab Instruments GmbH & Co. KG, Otto-Brenner-Straße 20, Göttingen, Germany), conjugate pad (10 mm wide, No. 6613, Ahlstrom-Munksjö, Oyj, Finland), sample pad (18 mm wide, Cat. No. 1281, Ahlstrom-Munksjö), wicking pad (14 mm wide, Cat. No. 440, Ahlstrom-Munksjö), cover tape (13 mm wide, Cat. No. 300H2, 3M, St. Paul, MN, USA) and backing card (50 mm wide, Cat. No. KN2211, Kenosha, Schweitzerlaan, The Netherlands).

All primers and probes, purified 2019-nCoV_N RNA, and Hs_RPP30 human RNA were purchased from IDT (Coralville, IA, USA). The Research Use Only (RUO) QIAamp Viral Mini Kit for RNA extraction was purchased from Qiagen (Hilden, Germany). The qScript XLT 1-Step RT-qPCR ToughMix was purchased from QuantaBio (Beverly, MA, USA). Molecular biology grade water was purchased from Fisher Scientific (Waltham, MA, USA).

A total of nine de-identified samples were purchased from Medix (Lombard, IL, USA). These samples included six SARS-CoV-2 positives and three negatives. All samples were discarded and de-identified and therefore did not require IRB approval for use.

RT-qPCR for detection of COVID-19 and quantification of SARS-CoV-2 viral load
The COVID-19 status of clinical samples used in this work was determined in-house using a multiplex RT-qPCR for the N1, N2, and RP targets.[10] Briefly, 70 or 140 µL of sample were purified using the QIAamp Viral Mini Kit according to the manufacturer's protocol[11] and purified RNA was eluted in either 70 or 140 µL based on CDC recommendations.[12] The multiplexed reaction was performed using the qScript master mix from QuantaBio with N1 and RP primers and probe concentrations of 500 nM and 125 nM (final) and N2 primers and probe concentrations of 2000 nM and 500 nM (final). The probes used were N1-FAM, N2-AlexaFluor594, and RP-Cy5. For each reaction, 5 µL of sample was added to 15 µL of amplification mix. Samples were classified as positive if both N1 and N2 targets were detected with Ct values below 40 cycles.[13] Viral load was determined using a standard curve for the N1 target generated from purified 2019-nCoV_N RNA. The purified SARS-CoV-2 RNA was quantified in-house using the BioRad QX200 Digital Droplet PCR System.

Antigen selection using Octet
Antibody–antigen interactions were evaluated with an Octet® RED96 biolayer interferometry instrument (Molecular Devices, Sartorius AG, Göttingen, Germany). All measurements were performed in 96-well microplates (Greiner Bio-one, Frickenhausen, Germany) at ambient temperature. Antibodies were loaded at 25 nM in 1× Kinetics Buffer for 120 seconds and captured using AMC tips for mouse antibodies, AHC tips for humanized recombinant antibodies, and Protein A tips for rabbit antibodies. Materials for the Octet were purchased from Molecular Devices. New sensors were used for every reaction and no tip regeneration was performed.

Typical immobilization levels were 1 ± 0.2 nm for monoclonal antibodies, and 2 nm for rabbit polyclonal antibodies. Following the load step, all sensors were equilibrated to baseline for 120 seconds in 1× Kinetics Buffer. Association step was performed for 300 seconds with antigen at 100 nM quantity, followed by 300 second dissociation into 1× Kinetics buffer.

Antibody/antigen evaluation by SDS-PAGE
Antigens were evaluated for purity and size using SDS-PAGE. Concentration was measured for all proteins using BCA assay (Thermo Pierce cat. 23225). Samples were premixed NuPAGE™ LDS Sample Buffer (4×) (Thermo Pierce cat. NP0007) and heated at 70°C for 10 minutes. Gels with a 4-12% Bis-Tris gradient were used to achieve separation. Coomassie Imperial™ Protein Stain (Thermo Pierce cat. 24615) was used to visualize bands. Novex Sharp Pre-stained protein standard (Thermo Fisher scientific) was used as a molecular weight marker.

Latex bead conjugation
For both test and control line detection conjugates, 400 nm carboxylic blue latex beads (Cat. No. CAB400NM, Magsphere, Pasadena CA, USA) were washed three times with 0.1M MES buffer, pH 6. Then, latex beads were activated using EDC/NHS coupling reagents at 0.15 and 10 mg/mL respectively for 30 minutes. Afterwards, the blue latex particles were conjugated in 1× PBS, pH 7.2 to various anti-nucleocapsid antibodies at a w/w ratio of 20:1 and 10:1 (bead: antibody) for test and control line antibodies, respectively, for three hours. Finally, latex conjugates were quenched using 0.1M ethanolamine before being washed and blocked with 6% (w/v) casein, final concentration 1.2%, overnight. The latex conjugates were stored in buffer containing 50mM borate and 1% casein, pH 8.5. The latex conjugates were quantified using the spectrophotometer by measuring absorbance at 660 nm and comparing to absorbance of unconjugated beads.

LFA reagent deposition
Capture antibodies at 1 mg/mL in 1× PBS, pH 7.4 and 2.5% (w/v) sucrose were striped (ZX1010, BioDot, Irvine, CA, USA) on nitrocellulose CN95 and dried at 25°C for 30 min. The control line was striped at 0.75 mg/mL Donkey anti-Chicken IgY (Cat. No. 703-005-155, Jackson ImmunoResearch, West Grove, PA, USA). For antibody screening, the nitrocellulose was unblocked. The test and control lines were located at 8 mm and 13 mm from the upstream edge of the nitrocellulose membrane.

The conjugate pad was dip-coated with two blocking solutions. First, 6613 conjugate pads were soaked in a 0.05% (w/v) Tween-20 in diH$_2$O solution for 15–20 seconds and dried at 40°C for 60 min. Pads were again soaked in 50mM borate, pH 8.5; 0.25% (w/v) Triton X-100; 1% (w/v) Surfactant-10G; 1% (w/v) sucrose; and 6% (w/v) casein for another 15–20 seconds. The conjugate pad was dried for 60 min at 40°C before assembly.

LFA Assembly
Card assembly was performed on a clamshell laminator (Matrix 2210, Kinematic Automation, Sonora CA, USA). Pads were placed on the backing card in the following order: nitrocellulose, cover tape, conjugate pad, sample pad, wicking pad. Individual strips (3.3 mm wide) were cut with a Matrix 2360 sheet cutter (Kinematic Automation) and assembled in cassettes (proprietary design) using an assembly roller (YK725, Kinbio Tech Co., Shanghai, China).

Hamilton screening procedure
Antibody pairs were screened on an integrated robotic system[9] we have previously used to test antibody performance directly on nitrocellulose. In this system, the Hamilton STAR automated liquid handling robot (Hamilton Company, Reno, NV, USA), camera (IDS UI-1460SE-C-H detector with a Tamron M118FM16 lens) custom LFA holders, and custom control software developed in-house were combined to allow rapid screening of antibody pairs directly in LFA format. The robot used 8-channel pipetting for parallel application to LFAs and the camera for imaging. The custom LFA framework held a maximum of 96 LFA cassettes per robot run. The custom control software applied 1 µL of latex bead conjugate mix (0.15% anti-nucleocapsid -latex bead, 0.1% or 0.05% Chicken IgY latex bead in 50mM borate pH 8.5) to the conjugate pad in the LFA. After a 10-minute delay to let the conjugate mix dry, 75 µL of sample, nucleocapsid protein or buffer (2.5% BSA in PBST or 2.5% BSA and 1% IGEPAL in 1× PBS) was added to the sample pad. Images were acquired 20 minutes after sample addition. Four technical replicates were run for each antibody pair per sample type.

Screening recombinant antigens on LFAs
We conducted four rounds of testing using recombinant NP as the antigen target. The first, with the best-available available NP antigen, at 50 ng/mL. The second, with a subsequently determined preferable antigen, at 50 ng/mL. A third round, under the same conditions but with data-driven down-selection of antibody pairs, and the fourth, with 25 ng/mL. A complete list of all pairs screened from all rounds is in Table 1si (supp. info).

Screening clinical samples on LFAs

In-house RT-qPCR was performed on banked nasopharyngeal clinical samples to confirm infection status prior to LFA testing (Table 1). When testing clinical samples, test and control line conjugates were hand spotted prior to sample application. The test line conjugate was diluted to a final concentration of 0.10% and control line Chicken IgY conjugate to 0.15% in 50 mM Borate, pH 8.5. First, 1 µL of conjugate mixture was pipetted onto the conjugate pad and allowed to dry at ambient temp for 10 minutes prior to application of the sample. All samples were diluted 1:25 in sample buffer containing 2.5% BSA and 1% IGEPAL in 1x PBS. Samples were incubated on ice for 30 minutes prior to use. Second, 75 µL of each sample diluted in sample buffer was added to the conjugate pad and run at ambient conditions inside a biosafety cabinet for 20 minutes prior to being read in an LFA reader.

Table 1 | Banked samples were used to compare performance of select anti-nucleocapsid antibody pairs in LFAs. In total, six RT-qPCR-confirmed SARS-CoV-2 positives, three SARS-CoV-2 negatives, and two potential coronavirus cross-reactive samples were screened.

Patient ID / Cat. No.	Clinical SARS-CoV-2 NAAT Results (pos v. neg)	SARS-CoV-2 Viral Load (c/uL, using purified RNA, N1 gene)	1:25 dilution, viral load (c/uL)	Human RNA Load (c/uL, using purified RNA, RP gene)	Vendor
4175017	+	3.5E+08	1.4E+07	9.2E+00	Medix Biochemica
4187771	+	2.2E+08	8.8E+06	1.4E+00	Medix Biochemica
4186565	+	1.5E+08	6.1E+06	2.1E+00	Medix Biochemica
4184163	+	7.3E+07	2.9E+06	-	Medix Biochemica
4182846	+	1.9E+06	7.6E+04	1.2E+04	Medix Biochemica
4183188	+	8.4E+05	3.4E+04	5.7E+01	Medix Biochemica
4177740	-	-	-	-	Medix Biochemica
4182799	-	-	-	1.6E+05	Medix Biochemica
4184232	-	-	-	6.1E+03	Medix Biochemica
HCoV-NL63 coronavirus	n/a	n/a	n/a	n/a	Abcam
HCoV-229E lysate VR740™	n/a	n/a	n/a	n/a	ATCC

Data analysis

Image analysis for the integrated robotic system was performed with a custom Python-based tool developed in-house.[9] This tool identified the test and control lines, measured nitrocellulose background intensity, and reported signal from the height of the line peak. Faulty LFAs were identified by low control line signal or poor shape and removed as outliers, however outlier removal was rare, occurring in fewer than 2% of all LFAs tested. The results were analyzed by calculating the average response for antigen positive samples, antigen negative samples, and the ratio and difference between these two signals.

Antibody pair rankings were determined by quantifying signal intensity divided by background noise (non-specific binding) and signal intensity subtracted by background noise-. Both metrics were used to increase the requirements of the best pairs to have both high positive control signal and low negative control signal. Four technical replicates were measured for all LFAs in the robotic screen portion of this work.

For benchtop analysis of LFAs, test and control line intensities were quantified using a LED-based LFA reader (Axxin, Fairfield, Australia).

Results and Discussion

Biolayer interferometry was performed on recombinant nucleocapsid proteins (NPs), for the purpose of selecting the most "native-like" analyte for LFA antibody screening. Initially, we used the estimated R_{max} of five different NPs to quantify binding affinity against a random selection of 21 anti-nucleocapsid antibodies from seven different vendors (Rockland, Novus Biologicals, Sino Biological, Creative Diagnostics, Bioss, Fitzgerald, and MyBiosource). The metric R_{max} was calculated based on theoretically saturating 100% of the bound antibody (ligand) with the analyte (NP). In practice, analyte binding sites are not completely occupied, so the measured saturation value is typically less than R_{max}. Moreover, because R_{max} is proportional to analyte size, we were also able to detect aggregation or multimer formation in solution. In theory, the closer—and more predictable—measured values were to R_{max} the more likely the antigen was to interact with antibodies as expected. The NP antigen from Genemedi was selected as the starting antigen for antibody screening because the average saturation value across 21 different anti-NP antibodies was closest to the theoretical R_{max} of the antigen (data not provided).

Round 1 of antibody pair screening on LFAs consisted of a 11 × 11 grid of antibodies (121 unique pairs). For each pair, one antibody was striped on nitrocellulose as a test line (the "capture" antibody) and the other was coupled to latex nanoparticles using EDC/NHS chemistry (the "detector" antibody). The results of the first round are given in Figure 1(A). The positive control for round 1 was 50 ng/mL NP from Genemedi. The negative control was 2.5% BSA in PBST. The top five antibody pairs after round 1 for both S/N and S-N were index pairs 540, 567, 564, 604, and 603 (Table 2). As anticipated, self-pairs did not perform well compared to non-self-pairs because Genemedi's nucleocapsid protein was monomeric and therefore likely to only contain a single copy of the sequence targeted by antibodies in the screen. Competition for the same epitope likely reduced the number of complete sandwich formation at the test line. Octet analysis also confirmed poor self-pair performance (data not shown). After completing round 1, 75 pairs were eliminated from further evaluation. To maintain a large antibody pair pool for subsequent rounds, any pair in the top 20 for S-N or S/N were re-screened in round 2, along with three new anti-NP antibodies.

The grid for round 2 was 11 × 11 (121 pairs); every antibody was evaluated as both capture and detectors. Results from round 2 are in Figure 1(B). The positive NP control was 50 ng/mL from Acro Biosystems. The negative control was 2.5% BSA in PBST. A new NP vendor was used for round 2 because we observed more consistent antibody binding (Octet measurement of binding saturation relative to R_{max}, data not provided) against a random selection of anti-NP antibodies when compared head-to-head with the Genemedi NP antigen used in round 1. Additionally, the antigen from Acro Biosystems was expressed in HEK293T cells whereas Genemedi's NP was *E. coli* produced; therefore, the mammalian cell expressed protein was most likely to display the biologically-relevant glycosylation patterns that viral proteins from infected human cells would express. Based on S/N and S-N metrics, the five best performing antibody pairs from round 2 were 33, 355, 653, 7, and 533 (Table 2). All five pairs from round 1 were in the top 60% of performers in round 2, and in total, 114 pairs were eliminated in round 2 from further examination.

Round 3 of screening contained seven pairs from round 2 and seven new anti-NP antibodies, again evaluated as both capture and detectors. By the third round, seven antibody pairs were producing strong signal intensity at the test line at 50 ng/mL, so another decision was made to reduce concentration of the Acro Biosystems NP antigen from 50 to 25 ng/mL to increase selectivity and emphasize the highest-performing pairs. The grid for round 3 was 12 × 12 (144 pairs). Results are displayed in Figure 1(C). The top five performers from round 3 by S/N and S-N were index pairs 533, 70, 50, 7, and 33 (Table 2). Three pairs (7, 533, and 33) were top-five performers from a previous round. Another 114 pairs were eliminated from further examination and 30 pairs were re-evaluated in round 4.

Antigen concentration for round 4 was decreased again from 25 to 10 ng/mL. The antibody pair grid size was 18 × 18 (324 pairs) to accommodate 12 new anti-NP antibodies. The top five performing pairs against the Acro Biosystems NP were 423, 33, 70, 422, and 403 (Table 2, Figure 1(D)). Pairs 33 and 70 were again repeated from earlier rounds, indicating that antibodies in these pairs had high affinity for the antigen from Acro Biosystems.

After concluding four rounds of screening, 673 unique anti-NP pairs had been screened with a combination of antigens from two different vendors (Genemedi and Acro Biosystems) and three different spike concentrations (50, 25, and 10 ng/mL), which was necessary because the average pair performance was reaching the non-linear peak of test line intensity. A complete list of all pairs screened are indexed in Table 1si (supp. info).
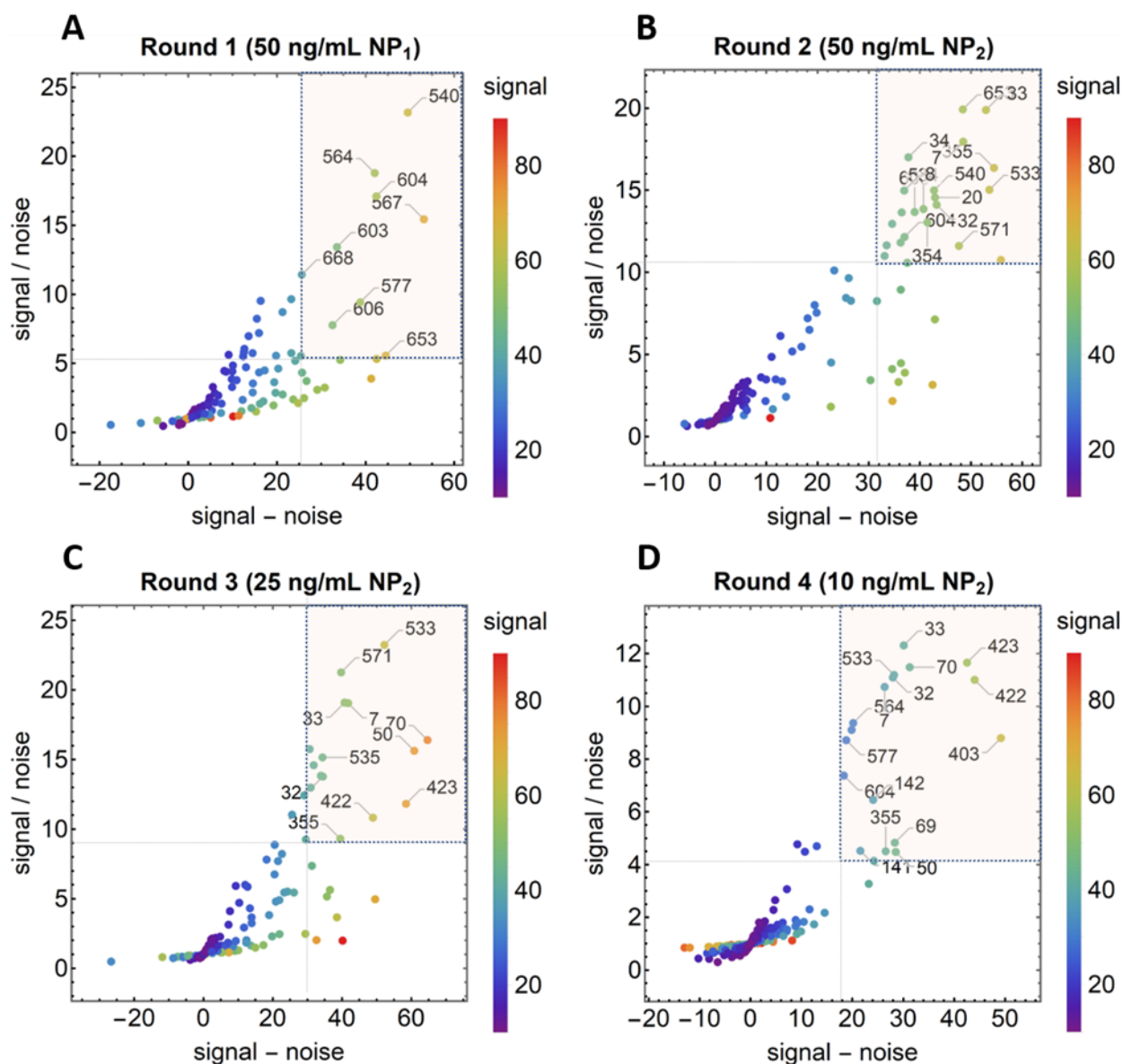
Figure 1 | A Performance of 673 individual antibody pairs in 4 rounds of screening as a function of signal / noise and signal - noise. Line intensities are shown as scatter plots for round 1 (**A**), round 2 (**B**), round 3 (**C**), and round 4 (**D**). Antibody pairs in the top 20 for both S/N and S-N are overlaid with a semi-transparent box and numbered by their index (full list in Table 1si). NP$_1$ antigen was sourced from GeneMedi and NP$_2$ antigen was sourced from Acro Biological.

Table 2 | Antibody pairs in the top 20 for both S/N and S-N are ranked according to the round in which they were tested. Pair 33 performed in the top 5 for rounds 2, 3, 4. Pairs 7 and 533 in top 5 for rounds 2, 3. Pair 70 in top 5 for rounds 3, 4. Table 1si (supp. info) contains a complete list of all pairs screened.

Index	Capture antibody	Detector antibody	Average rank rd. 1	rd. 2	rd. 3	rd. 4
Top 5 performers (round 1)						
540	Sino Biological 40143-MM08	Creative Diagnostics DCABH-4693	1.5	9	-	-
567	Sino Biological 40143-MM08	Sino Biological 40143-R004	2.5	25	-	-
564	Sino Biological 40143-MM08	Sino Biological 40143-MM05	4	21 .5	14	12
604	Sino Biological 40143-R001	Sino Biological 40143-MM08	4	17.5	25	15.5
603	Sino Biological 40143-R001	Sino Biological 40143-MM05	7.5	13.5	19	13
Top 5 performers (round 2)						
33	Bioss bsm-41411M	Sino Biological 40143-MM08	-	3	5.5	3
355	Fitzgerald 10-2856	Sino Biological 40143-MM08	-	3.5	14	14.5
653	Sino Biological 40143-R040	Sino Biological 40143-MM08	11.5	3.5	16.5	-
7	Bioss bsm-41411M	Creative Diagnostics CABT-CS037	-	4	5.5	8.5
533	Sino Biological 40143-MM08	Bioss bsm-41411M	-	4.5	2.5	6
Top 5 performers (round 3)						
533	Sino Biological 40143-MM08	Bioss bsm-41411M	-	4.5	2.5	6
70	Bioss bsm-41413M	Sino Biological 40143-MM08	-	-	3	3.5
50	Bioss bsm-41413M	Creative Diagnostics CABT-CS037	-	-	4.5	13
7	Bioss bsm-41411M	Creative Diagnostics CABT-CS037	-	4	5.5	8.5
33	Bioss bsm-41411M	Sino Biological 40143-MM08	-	3	5.5	3
Top 5 performers (round 4)						
423	Genemedi GMP-V-2019nCoV-NAb001	Sino Biological 40143-MM08	-	-	8.5	2.5
33	Bioss bsm-41411M	Sino Biological 40143-MM08	-	3	5.5	3
70	Bioss bsm-41413M	Sino Biological 40143-MM08	-	-	3	3.5
422	Genemedi GMP-V-2019nCoV-NAb001	Sino Biological 40143-MM05	-	-	11	4
403	Genemedi GMP-V-2019nCoV-NAb00	Creative Diagnostics CABT-CS037	-	-	19	5.5

Another important feature of screening large numbers of antibody pairs in an LFA format is the ability to identify pairs that non-specifically bind at the test line. The unique interplay of flow dynamics and chemical kinetics across reagents and materials in an LFA means that screening data from non-LFA formats sometimes does not predict non-specific binding in an LFA format. We have found that screening data from the high-throughput robotic platform does predict non-specific binding in the LFA even when screened with different sample matrices, such as clinical negatives at multiple dilutions (Figure 2si). Additionally, several rounds of negative sample screening data can often be combined—even if positive samples are varied across rounds—if the negative samples are consistent across rounds, as was the case here. Combined negative sample data was used to remove pairs from contention when non-specific binding was greater than a self-defined threshold (e.g. a nominal specificity target), which was helpful because the number of pairs was large. This method reduced the likelihood that a high positive signal was primarily driven by non-specific binding and performing well artificially.

To demonstrate the difference between pairs identified as *high, moderate, and low*-performers, we selected 16 pairs with which to screen banked clinical SARS-CoV-2 positive, negative, and potentially cross-reactive samples. The two cross-reactive samples tested were confirmed positive for non-SARS-CoV-2 coronavirus (types 229E and NL63). No addition optimization of the LFA was performed beyond basic steps such as blocking the conjugate pad. Results from the clinical screen are shown in Figure 2. The top and bottom charts measure performance as a function of S-N and S/N, respectively. Signals were derived from three technical replicates on up to four positive clinical samples. Noise was pooled from three technical replicates across a blank sample and/or up to three negative clinical samples. Two additional positive clinical samples were tested but showed little-to-no response across all pairs and were excluded from the analysis. Finally, the S-N and S/N results corresponding to each positive sample were normalized by the logarithm of the viral load in each positive sample to allow for a more accurate performance comparison across test conditions.

The data showed that the best pairs (e.g. index pairs 567, 527) were at least 15-fold higher in S-N intensity, on average, across all positive samples when compared with LFA pairs identified in the screen as poor performers (e.g. index pairs 666, 517). Signal intensities varied for different clinical positives, as expected, however 2/6 samples (IDs 846 and 188, Table 1) were not visible on any LFA and were therefore excluded from analysis. A complete dataset is provided in Figure 1si (supp. info). After dilution, the viral load of these two samples was 3–7×10^4 (c/µL), indicating the LOD of these LFAs, without additional optimization is roughly 1×10^5 c/µL. A previous paper from our group reported the optimization of a half-strip LFA targeting SARS-CoV-2 viral NP.[14] There was no visible non-specific binding or cross-reactivity to related coronavirus samples 229E and NL63 (Figure 1, supp. info), but additional screening of potential cross-reactivity should be performed on candidate pairs. The LFAs that performed the best against clinical positive, negative, and potentially reactive samples used antibody index pairs 567, 527, 564, and 111 (Table 3).
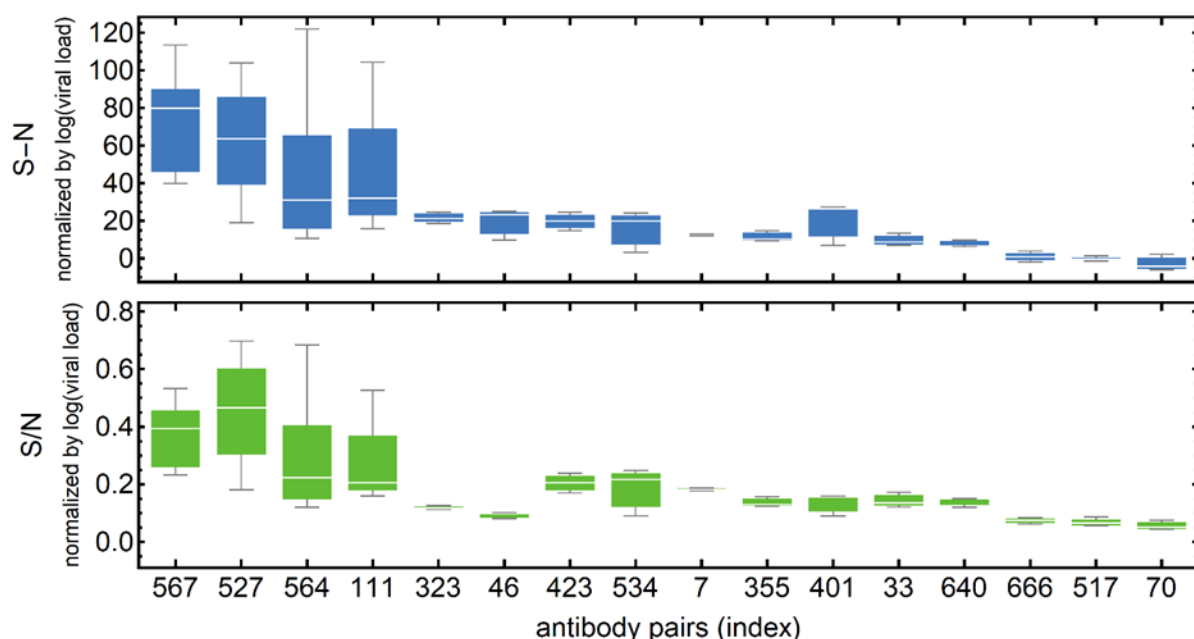
Figure 2 | Performance of 16 selected antibody pairs on clinical samples as a function of signal – noise (S-N) (TOP graph) and signal / noise (S/N) (BOTTOM graph).

Interestingly, pairs testing well in rounds 2–4 wherein NP from Acro Biosystems was the target did not perform as well as expected in the clinical screen. Table 3si in supp. info ranks pairs from the clinical screen as well as each pair's ranking (avg. of S/N and S-N) in rounds 1–4. Index pairs 33, 70, 7, and 423, for example, were top-10 performers in one or more rounds, however in the clinical screening round, the average S-N intensity across all positive samples was 75-94% *lower* than the best performing pair (index pair 567). Specific antibodies (e.g. Bioss bsm-41411M) appeared to have higher affinity for the antigen from Acro Biosystems but performed below expectation when were included in pairs that were tested against banked clinical samples pairs contained this antibody underperformed expectations. The *E. coli* produced antigen from Genemedi appeared to best predict antibody pair performance against clinical samples, however additional investigation is warranted.

Table 3 | Antibody pairs selected to be screened against clinical samples are ranked according to average performance by S-N and S/N in the clinical screen. Table 3si (supp. info.) includes a full list of average rankings from all four high-throughput robotic platform screening rounds.

Index	Capture antibody	Detector antibody	Avg. Rank
567	Sino Biological 40143-MM08	Sino Biological 40143-R004	1
527	Sino Biological 40143-MM05	Sino Biological 40143-MM08	2
564	Sino Biological 40143-MM08	Sino Biological 40143-MM05	3
111	Creative Diagnostics CABT-CS037	Sino Biological 40143-R004	4.5
423	Genemedi GMP-V-2019nCoV-NAb001	Sino Biological 40143-MM08	5.5
7	Bioss bsm-41411M	Creative Diagnostics CABT-CS037	7.5
534	Sino Biological 40143-MM08	Bioss bsm-41412M	7.5
323	Fitzgerald 10-2856	Bioss bsm-41411M	8
46	Bioss bsm-41413M	Bioss bsm-41411M	9.5
355	Fitzgerald 10-2856	Sino Biological 40143-MM08	9.5
640	Sino Biological 40143-R040	Creative Diagnostics CABT-CS037	10.5
33	Bioss bsm-41411M	Sino Biological 40143-MM08	11
401	Genemedi GMP-V-2019nCoV-NAb001	Bioss bsm-41413M	11.5
517	Novus Bio NB100-56683	Sino Biological 40143-MM05	14.5
666	Sino Biological 40588-T62	Novus Bio NB100-56683	14.5
70	Bioss bsm-41413M	Sino Biological 40143-MM08	16

Conclusions

Six hundred seventy-three antibody pairs were screened against SARS-CoV-2 nucleocapsid protein, and multiple candidates from several different commercially available sources were identified as promising candidates towards the development of lateral flow assays for the detection of SARS-CoV-2. Further work is required for the development of a point-of-care test for SARS-CoV-2, though the antibodies screened within this paper provide a necessary step towards its development. The antibody pairs that we identify as the top-ranking pairs should be interpreted as down-selected, though not necessarily precisely ordered list of the best potential candidates for developing an LFA. We suggest that multiple of the top pairs we identified be tested further by anyone attempting to develop an LFA using these data, as the precise interaction of all assay components, materials, and methods can affect which pair will perform optimally.

Acknowledgements

References

(1)   Zhu, N.; Zhang, D.; Wang, W.; Li, X.; Yang, B.; Song, J.; Zhao, X.; Huang, B.; Shi, W.; Lu, R.; Niu, P.; Zhan, F.; Ma, X.; Wang, D.; Xu, W.; Wu, G.; Gao, G. F.; Tan, W. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N. Engl. J. Med.* **2020**, *382* (8), 727–733. https://doi.org/10.1056/NEJMoa2001017.

(2)   Coronavirus disease (COVID-19) Situation Report https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200723-covid-19-sitrep-185.pdf?sfvrsn=9395b7bf_2 (accessed Jul 23, 2020).

(3)   Larremore, D. B.; Wilder, B.; Lester, E.; Shehata, S.; Burke, J. M.; Hay, J. A.; Tambe, M.; Mina, M. J.; Parker, R. Test Sensitivity Is Secondary to Frequency and Turnaround Time for COVID-19 Surveillance. *medRxiv* **2020**, 2020.06.22.20136309. https://doi.org/10.1101/2020.06.22.20136309.

(4)   He, X.; Lau, E. H. Y.; Wu, P.; Deng, X.; Wang, J.; Hao, X.; Lau, Y. C.; Wong, J. Y.; Guan, Y.; Tan, X.; Mo, X.; Chen, Y.; Liao, B.; Chen, W.; Hu, F.; Zhang, Q.; Zhong, M.; Wu, Y.; Zhao, L.; Zhang, F.; Cowling, B. J.; Li, F.; Leung, G. M. Temporal Dynamics in Viral Shedding and Transmissibility of COVID-19. *Nat. Med.* **2020**, *26* (5), 672–675. https://doi.org/10.1038/s41591-020-0869-5.

(5)   Alexandersen, S.; Chamings, A.; Bhatta, T. R. SARS-CoV-2 Genomic and Subgenomic RNAs in Diagnostic Samples Are Not an Indicator of Active Replication. *medRxiv* **2020**, 2020.06.01.20119750. https://doi.org/10.1101/2020.06.01.20119750.

(6)   Sofia SARS Antigen FIA https://www.fda.gov/media/137885/download (accessed Jul 23, 2020).

(7)   Veritor System For Rapid Detection of SARS-CoV-2 https://www.fda.gov/media/139755/download (accessed Jul 23, 2020).

(8)   Expression of interest - FIND https://www.finddx.org/eoi-covid19-ag-rdt/ (accessed Jul 23, 2020).

(9)   Huynh, T.; Cate, D. M.; Nichols, K. P.; Weigl, B. H.; Anderson, C. E.; Gasperino, D. J.; Harston, S. P.; Hsieh, H. V.; Marzan, R.; Williford, J. R.; Oncina, C. I.; Glukhova, V. A. Integrated Robotic System for the Development Lateral Flow Assays. In *2019 IEEE Global Humanitarian Technology Conference, GHTC 2019*; Institute of Electrical and Electronics Engineers Inc., 2019. https://doi.org/10.1109/GHTC46095.2019.9033066.

(10)   Byrnes, S. A.; Gallagher, R.; Steadman, A.; Bennett, C.; Rivera, R.; Ortega, C.; Motley, S. T.; Jain, P.; Weigl, B. H.; Connelly, J. T. Multiplexed and Extraction-Free Amplification for Simplified SARS-CoV-2 RT-PCR Tests. *medRxiv* **2020**, 2020.05.21.20106195. https://doi.org/10.1101/2020.05.21.20106195.

(11)   QIAamp Viral RNA Mini Handbook - QIAGEN https://www.qiagen.com/us/resources/resourcedetail?id=c80685c0-4103-49ea-aa72-8989420e3018&lang=en (accessed Jul 23, 2020).

(12)   *CDC 2019-Novel Coronavirus (2019-NCoV) Real-Time RT-PCR Diagnostic Panel For Emergency Use Only Instructions for Use*.

(13)   Bustin, S. A.; Benes, V.; Garson, J. A.; Hellemans, J.; Huggett, J.; Kubista, M.; Mueller, R.; Nolan, T.; Pfaffl, M. W.; Shipley, G. L.; Vandesompele, J.; Wittwer, C. T. The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. *Clin. Chem.* **2009**, *55* (4), 611–622. https://doi.org/10.1373/clinchem.2008.112797.

(14)   Grant, B. D.; Anderson, C. E.; Williford, J. R.; Alonzo, L. F.; Glukhova, V. A.; Boyle, D. S.; Weigl, B. H.; Nichols, K. P. A SARS-CoV-2 Coronavirus Nucleocapsid Antigen-Detecting Half-Strip Lateral Flow Assay Towards the Development of Point of Care Tests Using Commercially Available Reagents. *Anal. Chem.* **2020**. https://doi.org/10.1021/acs.analchem.0c01975.

Supplemental Information: Antibody Screening Results for Anti-Nucleocapsid Antibodies Towards the Development of a SARS-CoV-2 Nucleocapsid Protein Antigen Detecting Lateral Flow Assay

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Table 1si (continued) | Antibody pairs screened in rounds 1-4 on LFAs.

Table 1si | Indexed list of antibody pairs screened in rounds 1-4 on LFAs.

Index	Capture antibody	Detector antibody	round 1	round 2	round 3	round 4
1	Bioss bsm-41411M	Bioss bsm-41411M	-	74.5	82.5	150.5
2	Bioss bsm-41411M	Bioss bsm-41412M	-	11	28.5	-
3	Bioss bsm-41411M	Bioss bsm-41413M	-	-	21	-
4	Bioss bsm-41411M	Bioss bsm-41414M	-	-	60	-
5	Bioss bsm-41411M	Bioss bsm-41415M	-	61.5	-	-
6	Bioss bsm-41411M	Creative Diagnostics CABT-RM320	-	65	-	-
7	Bioss bsm-41411M	Creative Diagnostics CABT-CS037	-	4	5.5	9
8	Bioss bsm-41411M	Creative Diagnostics DCABH-4693	-	14	-	-
9	Bioss bsm-41411M	East Coast Bio HM1054	-	-	-	22.5
10	Bioss bsm-41411M	East Coast Bio HM1055	-	-	-	35.5
11	Bioss bsm-41411M	East Coast Bio HM1056	-	-	-	199
12	Bioss bsm-41411M	East Coast Bio HM1057	-	-	-	224
13	Bioss bsm-41411M	East Coast Bio HM1058	-	-	-	88.5
14	Bioss bsm-41411M	East Coast Bio HM1063	-	-	-	160
15	Bioss bsm-41411M	East Coast Bio HM1064	-	-	-	102
16	Bioss bsm-41411M	East Coast Bio HM1065	-	-	-	131.5
17	Bioss bsm-41411M	East Coast Bio HM1066	-	-	-	102.5
18	Bioss bsm-41411M	East Coast Bio HM1068	-	-	-	266.5
19	Bioss bsm-41411M	East Coast Bio HM1069	-	-	-	194
20	Bioss bsm-41411M	Fitzgerald 10-2856	-	9	-	-
21	Bioss bsm-41411M	Fitzgerald 10-2857	-	37.5	-	-
22	Bioss bsm-41411M	Fitzgerald 10-2860	-	-	-	86.5
23	Bioss bsm-41411M	Fitzgerald 10-2861	-	-	106	-
24	Bioss bsm-41411M	Genemedi GMP-V-2019nCoV-NAb001	-	-	135.5	-
25	Bioss bsm-41411M	Genemedi GMP-V-2019nCoV-NAb002	-	-	51.5	-
26	Bioss bsm-41411M	Medix Bio 100531	-	-	-	258.5
27	Bioss bsm-41411M	Medix Bio 100532	-	-	-	270
28	Bioss bsm-41411M	MyBiosource MBS569937	-	65	-	-
29	Bioss bsm-41411M	MyBiosource MBS569939	-	143	-	-
30	Bioss bsm-41411M	MyBiosource MBS569951	-	122.5	-	-
31	Bioss bsm-41411M	MyBiosource MBS569961	-	-	105.5	-
32	Bioss bsm-41411M	Sino Biological 40143-MM05	-	9	13	7
33	Bioss bsm-41411M	Sino Biological 40143-MM08	-	3	5.5	3
34	Bioss bsm-41411M	Sino Biological 40143-R004	-	10	90.5	-
35	Bioss bsm-41412M	Bioss bsm-41411M	-	59	-	-
36	Bioss bsm-41412M	Bioss bsm-41412M	-	140	-	-
37	Bioss bsm-41412M	Bioss bsm-41415M	-	108	-	-
38	Bioss bsm-41412M	Creative Diagnostics CABT-CS037	-	112	-	-
39	Bioss bsm-41412M	Creative Diagnostics DCABH-4693	-	74	-	-
40	Bioss bsm-41412M	Fitzgerald 10-2856	-	86.5	-	-
41	Bioss bsm-41412M	Fitzgerald 10-2857	-	137	-	-
42	Bioss bsm-41412M	MyBiosource MBS569951	-	101	-	-
43	Bioss bsm-41412M	Sino Biological 40143-MM05	-	138	-	-
44	Bioss bsm-41412M	Sino Biological 40143-MM08	-	133	-	-
45	Bioss bsm-41412M	Sino Biological 40143-R004	-	99	-	-
46	Bioss bsm-41413M	Bioss bsm-41411M	-	-	36	23.5

Table 1si (continued) | Antibody pairs screened in rounds 1-4 on LFAs.

Index	Capture antibody	Detector antibody	round 1	round 2	round 3	round 4	Index	Capture antibody	Detector antibody	round 1	round 2	round 3	round 4
47	Bioss bsm-41413M	Bioss bsm-41412M	-	-	72	-	71	Bioss bsm-41413M	Sino Biological 40143-R004	-	-	115	-
48	Bioss bsm-41413M	Bioss bsm-41413M	-	-	90.5	-	72	Bioss bsm-41414M	Bioss bsm-41411M	-	-	94.5	-
49	Bioss bsm-41413M	Bioss bsm-41414M	-	-	42	-	73	Bioss bsm-41414M	Bioss bsm-41412M	-	-	72.5	-
50	Bioss bsm-41413M	Creative Diagnostics CABT-CS037	-	-	4.5	13	74	Bioss bsm-41414M	Bioss bsm-41413M	-	-	77.5	-
51	Bioss bsm-41413M	East Coast Bio HM1054	-	-	-	46	75	Bioss bsm-41414M	Bioss bsm-41414M	-	-	132	-
52	Bioss bsm-41413M	East Coast Bio HM1055	-	-	-	171.5	76	Bioss bsm-41414M	Creative Diagnostics CABT-CS037	-	-	75.5	-
53	Bioss bsm-41413M	East Coast Bio HM1056	-	-	-	51	77	Bioss bsm-41414M	Fitzgerald 10-2861	-	-	42	-
54	Bioss bsm-41413M	East Coast Bio HM1057	-	-	-	197.5	78	Bioss bsm-41414M	Genemedi GMP-V-2019nCoV-NAb001	-	-	81	-
55	Bioss bsm-41413M	East Coast Bio HM1058	-	-	-	73.5	79	Bioss bsm-41414M	Genemedi GMP-V-2019nCoV-NAb002	-	-	125	-
56	Bioss bsm-41413M	East Coast Bio HM1063	-	-	-	327	80	Bioss bsm-41414M	MyBiosource MBS569961	-	-	65	-
57	Bioss bsm-41413M	East Coast Bio HM1064	-	-	-	45	81	Bioss bsm-41414M	Sino Biological 40143-MM05	-	-	84	-
58	Bioss bsm-41413M	East Coast Bio HM1065	-	-	-	190.5	82	Bioss bsm-41414M	Sino Biological 40143-MM08	-	-	62.5	-
59	Bioss bsm-41413M	East Coast Bio HM1066	-	-	-	263	83	Bioss bsm-41414M	Sino Biological 40143-R004	-	-	84	-
60	Bioss bsm-41413M	East Coast Bio HM1068	-	-	-	315.5	84	Bioss bsm-41415M	Bioss bsm-41411M	-	81	-	-
61	Bioss bsm-41413M	East Coast Bio HM1069	-	-	-	296.5	85	Bioss bsm-41415M	Bioss bsm-41412M	-	56.5	-	-
62	Bioss bsm-41413M	Fitzgerald 10-2860	-	-	-	81.5	86	Bioss bsm-41415M	Bioss bsm-41415M	-	91	-	-
63	Bioss bsm-41413M	Fitzgerald 10-2861	-	-	134	-	87	Bioss bsm-41415M	Creative Diagnostics CABT-CS037	-	56	-	-
64	Bioss bsm-41413M	Genemedi GMP-V-2019nCoV-NAb001	-	-	128	-	88	Bioss bsm-41415M	Creative Diagnostics DCABH-4693	-	103	-	-
65	Bioss bsm-41413M	Genemedi GMP-V-2019nCoV-NAb002	-	-	38.5	-	89	Bioss bsm-41415M	Fitzgerald 10-2856	-	60.5	-	-
66	Bioss bsm-41413M	Medix Bio 100531	-	-	-	357	90	Bioss bsm-41415M	Fitzgerald 10-2857	-	98.5	-	-
67	Bioss bsm-41413M	Medix Bio 100532	-	-	-	281.5	91	Bioss bsm-41415M	MyBiosource MBS569951	-	145.5	-	-
68	Bioss bsm-41413M	MyBiosource MBS569961	-	-	138.5	-	92	Bioss bsm-41415M	Sino Biological 40143-MM05	-	84	-	-
69	Bioss bsm-41413M	Sino Biological 40143-MM05	-	-	20.5	10.5	93	Bioss bsm-41415M	Sino Biological 40143-MM08	-	67	-	-
70	Bioss bsm-41413M	Sino Biological 40143-MM08	-	-	3	3.5	94	Bioss bsm-41415M	Sino Biological 40143-R004	-	87.5	-	-
							95	Creative Diagnostics CABT-RM320	Creative Diagnostics CABT-RM320	82	-	-	-

Table 1si (continued) | Antibody pairs screened in rounds 1-4 on LFAs.

Index	Capture antibody	Detector antibody	Average rank				Index	Capture antibody	Detector antibody	Average rank			
			round 1	round 2	round 3	round 4				round 1	round 2	round 3	round 4
96	Creative Diagnostics CABT-RM320	Creative Diagnostics CABT-CS037	37	-	-	-	117	Creative Diagnostics DCABH-4693	Creative Diagnostics DCABH-4693	75.5	-	-	-
97	Creative Diagnostics CABT-RM320	Creative Diagnostics DCABH-4693	47	-	-	-	118	Creative Diagnostics DCABH-4693	Sino Biological 40143-MM05	45.5	-	-	-
98	Creative Diagnostics CABT-RM320	Sino Biological 40143-MM05	59.5	-	-	-	119	Creative Diagnostics DCABH-4693	Sino Biological 40143-MM08	31	-	-	-
99	Creative Diagnostics CABT-RM320	Sino Biological 40143-MM08	33.5	-	-	-	120	Creative Diagnostics DCABH-4693	Sino Biological 40143-R001	68.5	-	-	-
100	Creative Diagnostics CABT-RM320	Sino Biological 40143-R001	102.5	-	-	-	121	Creative Diagnostics DCABH-4693	Sino Biological 40143-R004	53.5	-	-	-
101	Creative Diagnostics CABT-RM320	Sino Biological 40143-R004	46	-	-	-	122	Creative Diagnostics DCABH-4693	Sino Biological 40143-R019	91	-	-	-
102	Creative Diagnostics CABT-RM320	Sino Biological 40143-R019	80.5	-	-	-	123	Creative Diagnostics DCABH-4693	Sino Biological 40143-R040	63.5	-	-	-
103	Creative Diagnostics CABT-RM320	Sino Biological 40143-R040	99	-	-	-	124	Creative Diagnostics DCABH-4693	Sino Biological 40588-T62	60	-	-	-
104	Creative Diagnostics CABT-RM320	Sino Biological 40588-T62	83	-	-	-	125	East Coast Bio HM1054	Bioss bsm-41411M	-	-	-	52.5
105	Creative Diagnostics CABT-CS037	Creative Diagnostics CABT-RM320	76.5	-	-	-	126	East Coast Bio HM1054	Creative Diagnostics CABT-CS037	-	-	-	27.5
106	Creative Diagnostics CABT-CS037	Creative Diagnostics CABT-CS037	77	-	-	-	127	East Coast Bio HM1054	East Coast Bio HM1054	-	-	-	317.5
107	Creative Diagnostics CABT-CS037	Creative Diagnostics DCABH-4693	28.5	-	-	-	128	East Coast Bio HM1054	East Coast Bio HM1055	-	-	-	247.5
108	Creative Diagnostics CABT-CS037	Sino Biological 40143-MM05	38	-	-	-	129	East Coast Bio HM1054	East Coast Bio HM1056	-	-	-	342.5
109	Creative Diagnostics CABT-CS037	Sino Biological 40143-MM08	80.5	-	-	-	130	East Coast Bio HM1054	East Coast Bio HM1057	-	-	-	272
110	Creative Diagnostics CABT-CS037	Sino Biological 40143-R001	30.5	-	-	-	131	East Coast Bio HM1054	East Coast Bio HM1058	-	-	-	327
111	Creative Diagnostics CABT-CS037	Sino Biological 40143-R004	46.5	-	-	-	132	East Coast Bio HM1054	East Coast Bio HM1063	-	-	-	158.5
112	Creative Diagnostics CABT-CS037	Sino Biological 40143-R019	70.5	-	-	-	133	East Coast Bio HM1054	East Coast Bio HM1064	-	-	-	103
113	Creative Diagnostics CABT-CS037	Sino Biological 40143-R040	43	-	-	-	134	East Coast Bio HM1054	East Coast Bio HM1065	-	-	-	211
114	Creative Diagnostics CABT-CS037	Sino Biological 40588-T62	56	-	-	-	135	East Coast Bio HM1054	East Coast Bio HM1066	-	-	-	324.5
115	Creative Diagnostics DCABH-4693	Creative Diagnostics CABT-RM320	75.5	-	-	-	136	East Coast Bio HM1054	East Coast Bio HM1068	-	-	-	308
116	Creative Diagnostics DCABH-4693	Creative Diagnostics CABT-CS037	24	-	-	-	137	East Coast Bio HM1054	East Coast Bio HM1069	-	-	-	47.5

Table 1si (continued) | Antibody pairs screened in rounds 1-4 on LFAs.

Index	Capture antibody	Detector antibody	round 1	round 2	round 3	round 4	Index	Capture antibody	Detector antibody	round 1	round 2	round 3	round 4
138	East Coast Bio HM1054	Fitzgerald 10-2860	-	-	-	307	159	East Coast Bio HM1055	Sino Biological 40143-MM05	-	-	-	264.5
139	East Coast Bio HM1054	Medix Bio 100531	-	-	-	302	160	East Coast Bio HM1055	Sino Biological 40143-MM08	-	-	-	119
140	East Coast Bio HM1054	Medix Bio 100532	-	-	-	57.5	161	East Coast Bio HM1056	Bioss bsm-41411M	-	-	-	114.5
141	East Coast Bio HM1054	Sino Biological 40143-MM05	-	-	-	16	162	East Coast Bio HM1056	Creative Diagnostics CABT-CS037	-	-	-	330.5
142	East Coast Bio HM1054	Sino Biological 40143-MM08	-	-	-	13	163	East Coast Bio HM1056	East Coast Bio HM1054	-	-	-	49
143	East Coast Bio HM1055	Bioss bsm-41411M	-	-	-	56.5	164	East Coast Bio HM1056	East Coast Bio HM1055	-	-	-	280.5
144	East Coast Bio HM1055	Creative Diagnostics CABT-CS037	-	-	-	340.5	165	East Coast Bio HM1056	East Coast Bio HM1056	-	-	-	262.5
145	East Coast Bio HM1055	East Coast Bio HM1054	-	-	-	282	166	East Coast Bio HM1056	East Coast Bio HM1057	-	-	-	235
146	East Coast Bio HM1055	East Coast Bio HM1055	-	-	-	238	167	East Coast Bio HM1056	East Coast Bio HM1058	-	-	-	153.5
147	East Coast Bio HM1055	East Coast Bio HM1056	-	-	-	330.5	168	East Coast Bio HM1056	East Coast Bio HM1063	-	-	-	101.5
148	East Coast Bio HM1055	East Coast Bio HM1057	-	-	-	234.5	169	East Coast Bio HM1056	East Coast Bio HM1064	-	-	-	332
149	East Coast Bio HM1055	East Coast Bio HM1058	-	-	-	267	170	East Coast Bio HM1056	East Coast Bio HM1065	-	-	-	54.5
150	East Coast Bio HM1055	East Coast Bio HM1063	-	-	-	104	171	East Coast Bio HM1056	East Coast Bio HM1066	-	-	-	159.5
151	East Coast Bio HM1055	East Coast Bio HM1064	-	-	-	168	172	East Coast Bio HM1056	East Coast Bio HM1068	-	-	-	189.5
152	East Coast Bio HM1055	East Coast Bio HM1065	-	-	-	128.5	173	East Coast Bio HM1056	East Coast Bio HM1069	-	-	-	161.5
153	East Coast Bio HM1055	East Coast Bio HM1066	-	-	-	223	174	East Coast Bio HM1056	Fitzgerald 10-2860	-	-	-	340
154	East Coast Bio HM1055	East Coast Bio HM1068	-	-	-	327.5	175	East Coast Bio HM1056	Medix Bio 100531	-	-	-	40.5
155	East Coast Bio HM1055	East Coast Bio HM1069	-	-	-	252	176	East Coast Bio HM1056	Medix Bio 100532	-	-	-	353
156	East Coast Bio HM1055	Fitzgerald 10-2860	-	-	-	138	177	East Coast Bio HM1056	Sino Biological 40143-MM05	-	-	-	262.5
157	East Coast Bio HM1055	Medix Bio 100531	-	-	-	63.5	178	East Coast Bio HM1056	Sino Biological 40143-MM08	-	-	-	49.5
158	East Coast Bio HM1055	Medix Bio 100532	-	-	-	76.5	179	East Coast Bio HM1057	Bioss bsm-41411M	-	-	-	53.5

Table 1si (continued) | Antibody pairs screened in rounds 1-4 on LFAs.

Index	Capture antibody	Detector antibody	Average rank round 1	round 2	round 3	round 4	Index	Capture antibody	Detector antibody	Average rank round 1	round 2	round 3	round 4
180	East Coast Bio HM1057	Creative Diagnostics CABT-CS037	-	-	-	89.5	201	East Coast Bio HM1058	East Coast Bio HM1056	-	-	-	302
181	East Coast Bio HM1057	East Coast Bio HM1054	-	-	-	289.5	202	East Coast Bio HM1058	East Coast Bio HM1057	-	-	-	263.5
182	East Coast Bio HM1057	East Coast Bio HM1055	-	-	-	181	203	East Coast Bio HM1058	East Coast Bio HM1058	-	-	-	309.5
183	East Coast Bio HM1057	East Coast Bio HM1056	-	-	-	127.5	204	East Coast Bio HM1058	East Coast Bio HM1063	-	-	-	328
184	East Coast Bio HM1057	East Coast Bio HM1057	-	-	-	221	205	East Coast Bio HM1058	East Coast Bio HM1064	-	-	-	316.5
185	East Coast Bio HM1057	East Coast Bio HM1058	-	-	-	162	206	East Coast Bio HM1058	East Coast Bio HM1065	-	-	-	296
186	East Coast Bio HM1057	East Coast Bio HM1063	-	-	-	324.5	207	East Coast Bio HM1058	East Coast Bio HM1066	-	-	-	80
187	East Coast Bio HM1057	East Coast Bio HM1064	-	-	-	118.5	208	East Coast Bio HM1058	East Coast Bio HM1068	-	-	-	278.5
188	East Coast Bio HM1057	East Coast Bio HM1065	-	-	-	275	209	East Coast Bio HM1058	East Coast Bio HM1069	-	-	-	125
189	East Coast Bio HM1057	East Coast Bio HM1066	-	-	-	115.5	210	East Coast Bio HM1058	Fitzgerald 10-2860	-	-	-	271.5
190	East Coast Bio HM1057	East Coast Bio HM1068	-	-	-	105	211	East Coast Bio HM1058	Medix Bio 100531	-	-	-	306.5
191	East Coast Bio HM1057	East Coast Bio HM1069	-	-	-	80	212	East Coast Bio HM1058	Medix Bio 100532	-	-	-	140.5
192	East Coast Bio HM1057	Fitzgerald 10-2860	-	-	-	168.5	213	East Coast Bio HM1058	Sino Biological 40143-MM05	-	-	-	160
193	East Coast Bio HM1057	Medix Bio 100531	-	-	-	308	214	East Coast Bio HM1058	Sino Biological 40143-MM08	-	-	-	327
194	East Coast Bio HM1057	Medix Bio 100532	-	-	-	230.5	215	East Coast Bio HM1063	Bioss bsm-41411M	-	-	-	179.5
195	East Coast Bio HM1057	Sino Biological 40143-MM05	-	-	-	113	216	East Coast Bio HM1063	Creative Diagnostics CABT-CS037	-	-	-	89.5
196	East Coast Bio HM1057	Sino Biological 40143-MM08	-	-	-	102	217	East Coast Bio HM1063	East Coast Bio HM1054	-	-	-	279.5
197	East Coast Bio HM1058	Bioss bsm-41411M	-	-	-	103	218	East Coast Bio HM1063	East Coast Bio HM1055	-	-	-	141
198	East Coast Bio HM1058	Creative Diagnostics CABT-CS037	-	-	-	214.5	219	East Coast Bio HM1063	East Coast Bio HM1056	-	-	-	274.5
199	East Coast Bio HM1058	East Coast Bio HM1054	-	-	-	290	220	East Coast Bio HM1063	East Coast Bio HM1057	-	-	-	156.5
200	East Coast Bio HM1058	East Coast Bio HM1055	-	-	-	179.5	221	East Coast Bio HM1063	East Coast Bio HM1058	-	-	-	243

Table 1si (continued) | Antibody pairs screened in rounds 1-4 on LFAs.

Index	Capture antibody	Detector antibody	round 1	round 2	round 3	round 4	Index	Capture antibody	Detector antibody	round 1	round 2	round 3	round 4
222	East Coast Bio HM1063	East Coast Bio HM1063	-	-	-	39.5	243	East Coast Bio HM1064	East Coast Bio HM1066	-	-	-	258.5
223	East Coast Bio HM1063	East Coast Bio HM1064	-	-	-	319	244	East Coast Bio HM1064	East Coast Bio HM1068	-	-	-	89
224	East Coast Bio HM1063	East Coast Bio HM1065	-	-	-	257	245	East Coast Bio HM1064	East Coast Bio HM1069	-	-	-	277
225	East Coast Bio HM1063	East Coast Bio HM1066	-	-	-	212.5	246	East Coast Bio HM1064	Fitzgerald 10-2860	-	-	-	48
226	East Coast Bio HM1063	East Coast Bio HM1068	-	-	-	323.5	247	East Coast Bio HM1064	Medix Bio 100531	-	-	-	181
227	East Coast Bio HM1063	East Coast Bio HM1069	-	-	-	314.5	248	East Coast Bio HM1064	Medix Bio 100532	-	-	-	37
228	East Coast Bio HM1063	Fitzgerald 10-2860	-	-	-	293	249	East Coast Bio HM1064	Sino Biological 40143-MM05	-	-	-	115.5
229	East Coast Bio HM1063	Medix Bio 100531	-	-	-	318	250	East Coast Bio HM1064	Sino Biological 40143-MM08	-	-	-	47.5
230	East Coast Bio HM1063	Medix Bio 100532	-	-	-	66	251	East Coast Bio HM1065	Bioss bsm-41411M	-	-	-	310.5
231	East Coast Bio HM1063	Sino Biological 40143-MM05	-	-	-	85.5	252	East Coast Bio HM1065	Creative Diagnostics CABT-CS037	-	-	-	263.5
232	East Coast Bio HM1063	Sino Biological 40143-MM08	-	-	-	221	253	East Coast Bio HM1065	East Coast Bio HM1054	-	-	-	341.5
233	East Coast Bio HM1064	Bioss bsm-41411M	-	-	-	56.5	254	East Coast Bio HM1065	East Coast Bio HM1055	-	-	-	87
234	East Coast Bio HM1064	Creative Diagnostics CABT-CS037	-	-	-	75.5	255	East Coast Bio HM1065	East Coast Bio HM1056	-	-	-	132
235	East Coast Bio HM1064	East Coast Bio HM1054	-	-	-	128	256	East Coast Bio HM1065	East Coast Bio HM1057	-	-	-	169.5
236	East Coast Bio HM1064	East Coast Bio HM1055	-	-	-	40	257	East Coast Bio HM1065	East Coast Bio HM1058	-	-	-	87.5
237	East Coast Bio HM1064	East Coast Bio HM1056	-	-	-	133.5	258	East Coast Bio HM1065	East Coast Bio HM1063	-	-	-	30
238	East Coast Bio HM1064	East Coast Bio HM1057	-	-	-	174	259	East Coast Bio HM1065	East Coast Bio HM1064	-	-	-	103.5
239	East Coast Bio HM1064	East Coast Bio HM1058	-	-	-	285	260	East Coast Bio HM1065	East Coast Bio HM1065	-	-	-	247.5
240	East Coast Bio HM1064	East Coast Bio HM1063	-	-	-	265	261	East Coast Bio HM1065	East Coast Bio HM1066	-	-	-	93
241	East Coast Bio HM1064	East Coast Bio HM1064	-	-	-	349	262	East Coast Bio HM1065	East Coast Bio HM1068	-	-	-	258.5
242	East Coast Bio HM1064	East Coast Bio HM1065	-	-	-	274.5	263	East Coast Bio HM1065	East Coast Bio HM1069	-	-	-	358

Table 1si (continued) | Antibody pairs screened in rounds 1-4 on LFAs.

Index	Capture antibody	Detector antibody	round 1	round 2	round 3	round 4	Index	Capture antibody	Detector antibody	round 1	round 2	round 3	round 4
				Average rank								**Average rank**	
264	East Coast Bio HM1065	Fitzgerald 10-2860	-	-	-	98.5	285	East Coast Bio HM1066	Sino Biological 40143-MM05	-	-	-	219
265	East Coast Bio HM1065	Medix Bio 100531	-	-	-	246	286	East Coast Bio HM1066	Sino Biological 40143-MM08	-	-	-	108.5
266	East Coast Bio HM1065	Medix Bio 100532	-	-	-	129.5	287	East Coast Bio HM1068	Bioss bsm-41411M	-	-	-	216.5
267	East Coast Bio HM1065	Sino Biological 40143-MM05	-	-	-	118.5	288	East Coast Bio HM1068	Creative Diagnostics CABT-CS037	-	-	-	309.5
268	East Coast Bio HM1065	Sino Biological 40143-MM08	-	-	-	84.5	289	East Coast Bio HM1068	East Coast Bio HM1054	-	-	-	158.5
269	East Coast Bio HM1066	Bioss bsm-41411M	-	-	-	212.5	290	East Coast Bio HM1068	East Coast Bio HM1055	-	-	-	201.5
270	East Coast Bio HM1066	Creative Diagnostics CABT-CS037	-	-	-	204	291	East Coast Bio HM1068	East Coast Bio HM1056	-	-	-	268
271	East Coast Bio HM1066	East Coast Bio HM1054	-	-	-	207	292	East Coast Bio HM1068	East Coast Bio HM1057	-	-	-	146.5
272	East Coast Bio HM1066	East Coast Bio HM1055	-	-	-	244.5	293	East Coast Bio HM1068	East Coast Bio HM1058	-	-	-	96.5
273	East Coast Bio HM1066	East Coast Bio HM1056	-	-	-	302.5	294	East Coast Bio HM1068	East Coast Bio HM1063	-	-	-	50
274	East Coast Bio HM1066	East Coast Bio HM1057	-	-	-	150	295	East Coast Bio HM1068	East Coast Bio HM1064	-	-	-	136
275	East Coast Bio HM1066	East Coast Bio HM1058	-	-	-	283	296	East Coast Bio HM1068	East Coast Bio HM1065	-	-	-	102
276	East Coast Bio HM1066	East Coast Bio HM1063	-	-	-	244.5	297	East Coast Bio HM1068	East Coast Bio HM1066	-	-	-	234
277	East Coast Bio HM1066	East Coast Bio HM1064	-	-	-	173.5	298	East Coast Bio HM1068	East Coast Bio HM1068	-	-	-	171
278	East Coast Bio HM1066	East Coast Bio HM1065	-	-	-	294.5	299	East Coast Bio HM1068	East Coast Bio HM1069	-	-	-	155.5
279	East Coast Bio HM1066	East Coast Bio HM1066	-	-	-	244	300	East Coast Bio HM1068	Fitzgerald 10-2860	-	-	-	36.5
280	East Coast Bio HM1066	East Coast Bio HM1068	-	-	-	182.5	301	East Coast Bio HM1068	Medix Bio 100531	-	-	-	327.5
281	East Coast Bio HM1066	East Coast Bio HM1069	-	-	-	104.5	302	East Coast Bio HM1068	Medix Bio 100532	-	-	-	137
282	East Coast Bio HM1066	Fitzgerald 10-2860	-	-	-	188	303	East Coast Bio HM1068	Sino Biological 40143-MM05	-	-	-	163
283	East Coast Bio HM1066	Medix Bio 100531	-	-	-	142.5	304	East Coast Bio HM1068	Sino Biological 40143-MM08	-	-	-	189
284	East Coast Bio HM1066	Medix Bio 100532	-	-	-	153.5	305	East Coast Bio HM1069	Bioss bsm-41411M	-	-	-	229

Table 1si (continued) | Antibody pairs screened in rounds 1-4 on LFAs.

Index	Capture antibody	Detector antibody	round 1	round 2	round 3	round 4	Index	Capture antibody	Detector antibody	round 1	round 2	round 3	round 4
306	East Coast Bio HM1069	Creative Diagnostics CABT-CS037	-	-	-	275.5	329	Fitzgerald 10-2856	Creative Diagnostics CABT-CS037	-	19.5	26	28.5
307	East Coast Bio HM1069	East Coast Bio HM1054	-	-	-	230	330	Fitzgerald 10-2856	Creative Diagnostics DCABH-4693	-	36.5	-	-
308	East Coast Bio HM1069	East Coast Bio HM1055	-	-	-	88.5	331	Fitzgerald 10-2856	East Coast Bio HM1054	-	-	-	64
309	East Coast Bio HM1069	East Coast Bio HM1056	-	-	-	338	332	Fitzgerald 10-2856	East Coast Bio HM1055	-	-	-	105.5
310	East Coast Bio HM1069	East Coast Bio HM1057	-	-	-	242	333	Fitzgerald 10-2856	East Coast Bio HM1056	-	-	-	309
311	East Coast Bio HM1069	East Coast Bio HM1058	-	-	-	315.5	334	Fitzgerald 10-2856	East Coast Bio HM1057	-	-	-	100.5
312	East Coast Bio HM1069	East Coast Bio HM1063	-	-	-	238	335	Fitzgerald 10-2856	East Coast Bio HM1058	-	-	-	231
313	East Coast Bio HM1069	East Coast Bio HM1064	-	-	-	307	336	Fitzgerald 10-2856	East Coast Bio HM1063	-	-	-	101.5
314	East Coast Bio HM1069	East Coast Bio HM1065	-	-	-	327.5	337	Fitzgerald 10-2856	East Coast Bio HM1064	-	-	-	218.5
315	East Coast Bio HM1069	East Coast Bio HM1066	-	-	-	195	338	Fitzgerald 10-2856	East Coast Bio HM1065	-	-	-	301
316	East Coast Bio HM1069	East Coast Bio HM1068	-	-	-	250	339	Fitzgerald 10-2856	East Coast Bio HM1066	-	-	-	335.5
317	East Coast Bio HM1069	East Coast Bio HM1069	-	-	-	302	340	Fitzgerald 10-2856	East Coast Bio HM1068	-	-	-	217.5
318	East Coast Bio HM1069	Fitzgerald 10-2860	-	-	-	245	341	Fitzgerald 10-2856	East Coast Bio HM1069	-	-	-	41
319	East Coast Bio HM1069	Medix Bio 100531	-	-	-	330.5	342	Fitzgerald 10-2856	Fitzgerald 10-2856	-	51	-	-
320	East Coast Bio HM1069	Medix Bio 100532	-	-	-	75	343	Fitzgerald 10-2856	Fitzgerald 10-2857	-	61.5	-	-
321	East Coast Bio HM1069	Sino Biological 40143-MM05	-	-	-	212	344	Fitzgerald 10-2856	Fitzgerald 10-2860	-	-	-	320
322	East Coast Bio HM1069	Sino Biological 40143-MM08	-	-	-	265.5	345	Fitzgerald 10-2856	Fitzgerald 10-2861	-	-	63.5	-
323	Fitzgerald 10-2856	Bioss bsm-41411M	-	43.5	32.5	44.5	346	Fitzgerald 10-2856	Genemedi GMP-V-2019nCoV-NAb001	-	-	104	-
324	Fitzgerald 10-2856	Bioss bsm-41412M	-	77.5	67.5	-	347	Fitzgerald 10-2856	Genemedi GMP-V-2019nCoV-NAb002	-	-	55.5	-
325	Fitzgerald 10-2856	Bioss bsm-41413M	-	-	38.5	-	348	Fitzgerald 10-2856	Medix Bio 100531	-	-	-	57.5
326	Fitzgerald 10-2856	Bioss bsm-41414M	-	-	70.5	-	349	Fitzgerald 10-2856	Medix Bio 100532	-	-	-	346
327	Fitzgerald 10-2856	Bioss bsm-41415M	-	39	-	-	350	Fitzgerald 10-2856	MyBiosource MBS569937	-	107.5	-	-
328	Fitzgerald 10-2856	Creative Diagnostics CABT-RM320	-	102.5	-	-	351	Fitzgerald 10-2856	MyBiosource MBS569939	-	103.5	-	-

Table 1si (continued) | Antibody pairs screened in rounds 1-4 on LFAs.

Index	Capture antibody	Detector antibody	round 1	round 2	round 3	round 4
352	Fitzgerald 10-2856	MyBiosource MBS569951	-	141	-	-
353	Fitzgerald 10-2856	MyBiosource MBS569961	-	137	137.5	-
354	Fitzgerald 10-2856	Sino Biological 40143-MM05	-	13.5	28.5	18
355	Fitzgerald 10-2856	Sino Biological 40143-MM08	-	3.5	14	14
356	Fitzgerald 10-2856	Sino Biological 40143-R004	-	33.5	75.5	-
357	Fitzgerald 10-2857	Bioss bsm-41415M	-	79	-	-
358	Fitzgerald 10-2857	Creative Diagnostics CABT-RM320	-	113	-	-
359	Fitzgerald 10-2857	Creative Diagnostics CABT-CS037	-	57.5	-	-
360	Fitzgerald 10-2857	Creative Diagnostics DCABH-4693	-	40.5	-	-
361	Fitzgerald 10-2857	Fitzgerald 10-2856	-	54.5	-	-
362	Fitzgerald 10-2857	Fitzgerald 10-2857	-	130	-	-
363	Fitzgerald 10-2857	MyBiosource MBS569937	-	89.5	-	-
364	Fitzgerald 10-2857	MyBiosource MBS569939	-	143	-	-
365	Fitzgerald 10-2857	MyBiosource MBS569961	-	128.5	-	-
366	Fitzgerald 10-2857	Sino Biological 40143-MM05	-	126	-	-
367	Fitzgerald 10-2857	Sino Biological 40143-MM08	-	59	-	-
368	Fitzgerald 10-2857	Sino Biological 40143-R004	-	64.5	-	-
369	Fitzgerald 10-2860	Bioss bsm-41411M	-	-	-	83
370	Fitzgerald 10-2860	Creative Diagnostics CABT-CS037	-	-	-	159.5
371	Fitzgerald 10-2860	East Coast Bio HM1054	-	-	-	308
372	Fitzgerald 10-2860	East Coast Bio HM1055	-	-	-	277
373	Fitzgerald 10-2860	East Coast Bio HM1056	-	-	-	75.5
374	Fitzgerald 10-2860	East Coast Bio HM1057	-	-	-	172
375	Fitzgerald 10-2860	East Coast Bio HM1058	-	-	-	84
376	Fitzgerald 10-2860	East Coast Bio HM1063	-	-	-	171.5
377	Fitzgerald 10-2860	East Coast Bio HM1064	-	-	-	221
378	Fitzgerald 10-2860	East Coast Bio HM1065	-	-	-	318.5
379	Fitzgerald 10-2860	East Coast Bio HM1066	-	-	-	295
380	Fitzgerald 10-2860	East Coast Bio HM1068	-	-	-	147
381	Fitzgerald 10-2860	East Coast Bio HM1069	-	-	-	36
382	Fitzgerald 10-2860	Fitzgerald 10-2860	-	-	-	302
383	Fitzgerald 10-2860	Medix Bio 100531	-	-	-	315.5
384	Fitzgerald 10-2860	Medix Bio 100532	-	-	-	355
385	Fitzgerald 10-2860	Sino Biological 40143-MM05	-	-	-	115.5
386	Fitzgerald 10-2860	Sino Biological 40143-MM08	-	-	-	172
387	Fitzgerald 10-2861	Bioss bsm-41411M	-	-	90	-
388	Fitzgerald 10-2861	Bioss bsm-41412M	-	-	137.5	-
389	Fitzgerald 10-2861	Bioss bsm-41413M	-	-	65	-
390	Fitzgerald 10-2861	Bioss bsm-41414M	-	-	128.5	-
391	Fitzgerald 10-2861	Creative Diagnostics CABT-CS037	-	-	66.5	-
392	Fitzgerald 10-2861	Fitzgerald 10-2861	-	-	118.5	-
393	Fitzgerald 10-2861	Genemedi GMP-V-2019nCoV-NAb001	-	-	139.5	-
394	Fitzgerald 10-2861	Genemedi GMP-V-2019nCoV-NAb002	-	-	128	-
395	Fitzgerald 10-2861	MyBiosource MBS569961	-	-	78	-
396	Fitzgerald 10-2861	Sino Biological 40143-MM05	-	-	75.5	-
397	Fitzgerald 10-2861	Sino Biological 40143-MM08	-	-	60.5	-
398	Fitzgerald 10-2861	Sino Biological 40143-R004	-	-	108	-

Table 1si (continued) | Antibody pairs screened in rounds 1-4 on LFAs.

Index	Capture antibody	Detector antibody	round 1	round 2	round 3	round 4
			Average rank			
399	Genemedi GMP-V-2019nCoV-NAb001	Bioss bsm-41411M	-	-	81.5	68
400	Genemedi GMP-V-2019nCoV-NAb001	Bioss bsm-41412M	-	-	34.5	-
401	Genemedi GMP-V-2019nCoV-NAb001	Bioss bsm-41413M	-	-	23	-
402	Genemedi GMP-V-2019nCoV-NAb001	Bioss bsm-41414M	-	-	51	-
403	Genemedi GMP-V-2019nCoV-NAb001	Creative Diagnostics CABT-CS037	-	-	19	5.5
404	Genemedi GMP-V-2019nCoV-NAb001	East Coast Bio HM1054	-	-	-	16.5
405	Genemedi GMP-V-2019nCoV-NAb001	East Coast Bio HM1055	-	-	-	24
406	Genemedi GMP-V-2019nCoV-NAb001	East Coast Bio HM1056	-	-	-	107
407	Genemedi GMP-V-2019nCoV-NAb001	East Coast Bio HM1057	-	-	-	129
408	Genemedi GMP-V-2019nCoV-NAb001	East Coast Bio HM1058	-	-	-	81.5
409	Genemedi GMP-V-2019nCoV-NAb001	East Coast Bio HM1063	-	-	-	301
410	Genemedi GMP-V-2019nCoV-NAb001	East Coast Bio HM1064	-	-	-	41.5
411	Genemedi GMP-V-2019nCoV-NAb001	East Coast Bio HM1065	-	-	-	289
412	Genemedi GMP-V-2019nCoV-NAb001	East Coast Bio HM1066	-	-	-	289
413	Genemedi GMP-V-2019nCoV-NAb001	East Coast Bio HM1068	-	-	-	153.5
414	Genemedi GMP-V-2019nCoV-NAb001	East Coast Bio HM1069	-	-	-	63
415	Genemedi GMP-V-2019nCoV-NAb001	Fitzgerald 10-2860	-	-	-	289.5
416	Genemedi GMP-V-2019nCoV-NAb001	Fitzgerald 10-2861	-	-	73.5	-
417	Genemedi GMP-V-2019nCoV-NAb001	Genemedi GMP-V-2019nCoV-NAb001	-	-	80.5	-
418	Genemedi GMP-V-2019nCoV-NAb001	Genemedi GMP-V-2019nCoV-NAb002	-	-	118.5	-
419	Genemedi GMP-V-2019nCoV-NAb001	Medix Bio 100531	-	-	-	31.5

Index	Capture antibody	Detector antibody	round 1	round 2	round 3	round 4
			Average rank			
420	Genemedi GMP-V-2019nCoV-NAb001	Medix Bio 100532	-	-	-	332.5
421	Genemedi GMP-V-2019nCoV-NAb001	MyBiosource MBS569961	-	-	134.5	-
422	Genemedi GMP-V-2019nCoV-NAb001	Sino Biological 40143-MM05	-	-	11	4
423	Genemedi GMP-V-2019nCoV-NAb001	Sino Biological 40143-MM08	-	-	8.5	2.5
424	Genemedi GMP-V-2019nCoV-NAb001	Sino Biological 40143-R004	-	-	33	-
425	Genemedi GMP-V-2019nCoV-NAb002	Bioss bsm-41411M	-	-	27.5	-
426	Genemedi GMP-V-2019nCoV-NAb002	Bioss bsm-41412M	-	-	55	-
427	Genemedi GMP-V-2019nCoV-NAb002	Bioss bsm-41413M	-	-	37	-
428	Genemedi GMP-V-2019nCoV-NAb002	Bioss bsm-41414M	-	-	123.5	-
429	Genemedi GMP-V-2019nCoV-NAb002	Creative Diagnostics CABT-CS037	-	-	34.5	-
430	Genemedi GMP-V-2019nCoV-NAb002	Fitzgerald 10-2861	-	-	123.5	-
431	Genemedi GMP-V-2019nCoV-NAb002	Genemedi GMP-V-2019nCoV-NAb001	-	-	130.5	-
432	Genemedi GMP-V-2019nCoV-NAb002	Genemedi GMP-V-2019nCoV-NAb002	-	-	100.5	-
433	Genemedi GMP-V-2019nCoV-NAb002	MyBiosource MBS569961	-	-	131.5	-
434	Genemedi GMP-V-2019nCoV-NAb002	Sino Biological 40143-MM05	-	-	36.5	-
435	Genemedi GMP-V-2019nCoV-NAb002	Sino Biological 40143-MM08	-	-	30	-
436	Genemedi GMP-V-2019nCoV-NAb002	Sino Biological 40143-R004	-	-	128	-
437	Medix Bio 100531	Bioss bsm-41411M	-	-	-	144.5
438	Medix Bio 100531	Creative Diagnostics CABT-CS037	-	-	-	311
439	Medix Bio 100531	East Coast Bio HM1054	-	-	-	226.5
440	Medix Bio 100531	East Coast Bio HM1055	-	-	-	316.5
441	Medix Bio 100531	East Coast Bio HM1056	-	-	-	192.5

Table 1si (continued) | Antibody pairs screened in rounds 1-4 on LFAs.

Index	Capture antibody	Detector antibody	Average rank round 1	round 2	round 3	round 4	Index	Capture antibody	Detector antibody	Average rank round 1	round 2	round 3	round 4
442	Medix Bio 100531	East Coast Bio HM1057	-	-	-	242.5	465	Medix Bio 100532	East Coast Bio HM1066	-	-	-	116.5
443	Medix Bio 100531	East Coast Bio HM1058	-	-	-	180.5	466	Medix Bio 100532	East Coast Bio HM1068	-	-	-	129
444	Medix Bio 100531	East Coast Bio HM1063	-	-	-	335.5	467	Medix Bio 100532	East Coast Bio HM1069	-	-	-	120.5
445	Medix Bio 100531	East Coast Bio HM1064	-	-	-	50	468	Medix Bio 100532	Fitzgerald 10-2860	-	-	-	148
446	Medix Bio 100531	East Coast Bio HM1065	-	-	-	70.5	469	Medix Bio 100532	Medix Bio 100531	-	-	-	320
447	Medix Bio 100531	East Coast Bio HM1066	-	-	-	279.5	470	Medix Bio 100532	Medix Bio 100532	-	-	-	138
448	Medix Bio 100531	East Coast Bio HM1068	-	-	-	196	471	Medix Bio 100532	Sino Biological 40143-MM05	-	-	-	213.5
449	Medix Bio 100531	East Coast Bio HM1069	-	-	-	115.5	472	Medix Bio 100532	Sino Biological 40143-MM08	-	-	-	212
450	Medix Bio 100531	Fitzgerald 10-2860	-	-	-	108	473	MyBiosource MBS569937	Bioss bsm-41411M	-	93.5	-	-
451	Medix Bio 100531	Medix Bio 100531	-	-	-	349.5	474	MyBiosource MBS569937	Bioss bsm-41412M	-	109.5	-	-
452	Medix Bio 100531	Medix Bio 100532	-	-	-	301.5	475	MyBiosource MBS569937	Bioss bsm-41415M	-	141	-	-
453	Medix Bio 100531	Sino Biological 40143-MM05	-	-	-	267	476	MyBiosource MBS569937	Creative Diagnostics CABT-CS037	-	98	-	-
454	Medix Bio 100531	Sino Biological 40143-MM08	-	-	-	245.5	477	MyBiosource MBS569937	Creative Diagnostics DCABH-4693	-	79.5	-	-
455	Medix Bio 100532	Bioss bsm-41411M	-	-	-	197.5	478	MyBiosource MBS569937	Fitzgerald 10-2856	-	87.5	-	-
456	Medix Bio 100532	Creative Diagnostics CABT-CS037	-	-	-	333.5	479	MyBiosource MBS569937	Fitzgerald 10-2857	-	76	-	-
457	Medix Bio 100532	East Coast Bio HM1054	-	-	-	259.5	480	MyBiosource MBS569937	MyBiosource MBS569951	-	115.5	-	-
458	Medix Bio 100532	East Coast Bio HM1055	-	-	-	133	481	MyBiosource MBS569937	Sino Biological 40143-MM05	-	74.5	-	-
459	Medix Bio 100532	East Coast Bio HM1056	-	-	-	109.5	482	MyBiosource MBS569937	Sino Biological 40143-MM08	-	84.5	-	-
460	Medix Bio 100532	East Coast Bio HM1057	-	-	-	206	483	MyBiosource MBS569937	Sino Biological 40143-R004	-	82.5	-	-
461	Medix Bio 100532	East Coast Bio HM1058	-	-	-	76	484	MyBiosource MBS569939	Bioss bsm-41411M	-	149.5	-	-
462	Medix Bio 100532	East Coast Bio HM1063	-	-	-	110	485	MyBiosource MBS569939	Bioss bsm-41415M	-	72.5	-	-
463	Medix Bio 100532	East Coast Bio HM1064	-	-	-	342	486	MyBiosource MBS569939	Creative Diagnostics CABT-CS037	-	121	-	-
464	Medix Bio 100532	East Coast Bio HM1065	-	-	-	298							

Table 1si (continued) | Antibody pairs screened in rounds 1-4 on LFAs.

Index	Capture antibody	Detector antibody	round 1	round 2	round 3	round 4	Index	Capture antibody	Detector antibody	round 1	round 2	round 3	round 4
487	MyBiosource MBS569939	Creative Diagnostics DCABH-4693	-	86	-	-	508	MyBiosource MBS569961	Fitzgerald 10-2861	-	-	138	-
488	MyBiosource MBS569939	Fitzgerald 10-2856	-	95	-	-	509	MyBiosource MBS569961	Genemedi GMP-V-2019nCoV-NAb001	-	-	133	-
489	MyBiosource MBS569939	Fitzgerald 10-2857	-	135	-	-	510	MyBiosource MBS569961	Genemedi GMP-V-2019nCoV-NAb002	-	-	94.5	-
490	MyBiosource MBS569939	MyBiosource MBS569951	-	101.5	-	-	511	MyBiosource MBS569961	MyBiosource MBS569951	-	118.5	-	-
491	MyBiosource MBS569939	Sino Biological 40143-MM05	-	95.5	-	-	512	MyBiosource MBS569961	MyBiosource MBS569961	-	-	112.5	-
492	MyBiosource MBS569939	Sino Biological 40143-MM08	-	64.5	-	-	513	MyBiosource MBS569961	Sino Biological 40143-MM05	-	55	102.5	-
493	MyBiosource MBS569939	Sino Biological 40143-R004	-	69.5	-	-	514	MyBiosource MBS569961	Sino Biological 40143-MM08	-	-	116.5	-
494	MyBiosource MBS569951	Creative Diagnostics CABT-RM320	-	107.5	-	-	515	MyBiosource MBS569961	Sino Biological 40143-R004	-	101	82	-
495	MyBiosource MBS569951	Creative Diagnostics CABT-CS037	-	105.5	-	-	516	Novus Bio NB100-56683	Novus Bio NB100-56683	91	-	-	-
496	MyBiosource MBS569951	Creative Diagnostics DCABH-4693	-	103	-	-	517	Novus Bio NB100-56683	Sino Biological 40143-MM05	87	-	-	-
497	MyBiosource MBS569951	MyBiosource MBS569937	-	97	-	-	518	Novus Bio NB100-56683	Sino Biological 40143-R001	104.5	-	-	-
498	MyBiosource MBS569951	MyBiosource MBS569939	-	143	-	-	519	Novus Bio NB100-56683	Sino Biological 40143-R019	100	-	-	-
499	MyBiosource MBS569951	Sino Biological 40143-MM05	-	127	-	-	520	Novus Bio NB100-56683	Sino Biological 40143-R040	96	-	-	-
500	MyBiosource MBS569951	Sino Biological 40143-MM08	-	118.5	-	-	521	Novus Bio NB100-56683	Sino Biological 40588-T62	100	-	-	-
501	MyBiosource MBS569951	Sino Biological 40143-R004	-	124.5	-	-	522	Sino Biological 40143-MM05	Creative Diagnostics CABT-RM320	67.5	-	-	-
502	MyBiosource MBS569961	Bioss bsm-41411M	-	145	144	-	523	Sino Biological 40143-MM05	Creative Diagnostics CABT-CS037	27.5	-	-	-
503	MyBiosource MBS569961	Bioss bsm-41412M	-	-	131.5	-	524	Sino Biological 40143-MM05	Creative Diagnostics DCABH-4693	24.5	-	-	-
504	MyBiosource MBS569961	Bioss bsm-41413M	-	-	109.5	-	525	Sino Biological 40143-MM05	Novus Bio NB100-56683	102.5	-	-	-
505	MyBiosource MBS569961	Bioss bsm-41414M	-	-	78.5	-	526	Sino Biological 40143-MM05	Sino Biological 40143-MM05	66.5	-	-	-
506	MyBiosource MBS569961	Creative Diagnostics CABT-CS037	-	-	85.5	-	527	Sino Biological 40143-MM05	Sino Biological 40143-MM08	30	-	-	-
507	MyBiosource MBS569961	Fitzgerald 10-2857	-	148	-	-	528	Sino Biological 40143-MM05	Sino Biological 40143-R001	19.5	-	-	-

Table 1si (continued) | Antibody pairs screened in rounds 1-4 on LFAs.

Index	Capture antibody	Detector antibody	round 1	round 2	round 3	round 4	Index	Capture antibody	Detector antibody	round 1	round 2	round 3	round 4
529	Sino Biological 40143-MM05	Sino Biological 40143-R004	29.5	-	-	-	550	Sino Biological 40143-MM08	East Coast Bio HM1068	-	-	-	177.5
530	Sino Biological 40143-MM05	Sino Biological 40143-R019	60	-	-	-	551	Sino Biological 40143-MM08	East Coast Bio HM1069	-	-	-	292
531	Sino Biological 40143-MM05	Sino Biological 40143-R040	37.5	-	-	-	552	Sino Biological 40143-MM08	Fitzgerald 10-2856	-	20.5	-	-
532	Sino Biological 40143-MM05	Sino Biological 40588-T62	43	-	-	-	553	Sino Biological 40143-MM08	Fitzgerald 10-2857	-	48	-	-
533	Sino Biological 40143-MM08	Bioss bsm-41411M	-	4.5	2.5	6	554	Sino Biological 40143-MM08	Fitzgerald 10-2860	-	-	-	309
534	Sino Biological 40143-MM08	Bioss bsm-41412M	-	12.5	27	-	555	Sino Biological 40143-MM08	Fitzgerald 10-2861	-	-	75	-
535	Sino Biological 40143-MM08	Bioss bsm-41413M	-	-	12	-	556	Sino Biological 40143-MM08	Genemedi GMP-V-2019nCoV-NAb001	-	-	52.5	-
536	Sino Biological 40143-MM08	Bioss bsm-41414M	-	-	43.5	-	557	Sino Biological 40143-MM08	Genemedi GMP-V-2019nCoV-NAb002	-	-	29.5	-
537	Sino Biological 40143-MM08	Bioss bsm-41415M	-	40	-	-	558	Sino Biological 40143-MM08	Medix Bio 100531	-	-	-	46.5
538	Sino Biological 40143-MM08	Creative Diagnostics CABT-RM320	24	67.5	-	-	559	Sino Biological 40143-MM08	Medix Bio 100532	-	-	-	105.5
539	Sino Biological 40143-MM08	Creative Diagnostics CABT-CS037	25.5	46	47	259.5	560	Sino Biological 40143-MM08	MyBiosource MBS569937	-	133	-	-
540	Sino Biological 40143-MM08	Creative Diagnostics DCABH-4693	1.5	9	-	-	561	Sino Biological 40143-MM08	MyBiosource MBS569939	-	83.5	-	-
541	Sino Biological 40143-MM08	East Coast Bio HM1054	-	-	-	18.5	562	Sino Biological 40143-MM08	MyBiosource MBS569951	-	116.5	-	-
542	Sino Biological 40143-MM08	East Coast Bio HM1055	-	-	-	136.5	563	Sino Biological 40143-MM08	MyBiosource MBS569961	-	-	104.5	-
543	Sino Biological 40143-MM08	East Coast Bio HM1056	-	-	-	213.5	564	Sino Biological 40143-MM08	Sino Biological 40143-MM05	4	21.5	14	12
544	Sino Biological 40143-MM08	East Coast Bio HM1057	-	-	-	163.5	565	Sino Biological 40143-MM08	Sino Biological 40143-MM08	35	43.5	40	60.5
545	Sino Biological 40143-MM08	East Coast Bio HM1058	-	-	-	244	566	Sino Biological 40143-MM08	Sino Biological 40143-R001	28	-	-	-
546	Sino Biological 40143-MM08	East Coast Bio HM1063	-	-	-	201	567	Sino Biological 40143-MM08	Sino Biological 40143-R004	2.5	25	86.5	-
547	Sino Biological 40143-MM08	East Coast Bio HM1064	-	-	-	141	568	Sino Biological 40143-MM08	Sino Biological 40143-R019	51	-	-	-
548	Sino Biological 40143-MM08	East Coast Bio HM1065	-	-	-	105.5	569	Sino Biological 40143-MM08	Sino Biological 40143-R040	38.5	-	-	-
549	Sino Biological 40143-MM08	East Coast Bio HM1066	-	-	-	110	570	Sino Biological 40143-MM08	Sino Biological 40588-T62	26.5	-	-	-

Table 1si (continued) | Antibody pairs screened in rounds 1-4 on LFAs.

Index	Capture antibody	Detector antibody	round 1	round 2	round 3	round 4
571	Sino Biological 40143-R001	Bioss bsm-41411M	-	13	6	21.5
572	Sino Biological 40143-R001	Bioss bsm-41412M	-	21	30	-
573	Sino Biological 40143-R001	Bioss bsm-41413M	-	-	72.5	-
574	Sino Biological 40143-R001	Bioss bsm-41414M	-	-	64	-
575	Sino Biological 40143-R001	Bioss bsm-41415M	-	52	-	-
576	Sino Biological 40143-R001	Creative Diagnostics CABT-RM320	93	123.5	-	-
577	Sino Biological 40143-R001	Creative Diagnostics CABT-CS037	8.5	23.5	14	14.5
578	Sino Biological 40143-R001	Creative Diagnostics DCABH-4693	15	29.5	-	-
579	Sino Biological 40143-R001	East Coast Bio HM1054	-	-	-	29.5
580	Sino Biological 40143-R001	East Coast Bio HM1055	-	-	-	56.5
581	Sino Biological 40143-R001	East Coast Bio HM1056	-	-	-	60.5
582	Sino Biological 40143-R001	East Coast Bio HM1057	-	-	-	205
583	Sino Biological 40143-R001	East Coast Bio HM1058	-	-	-	264
584	Sino Biological 40143-R001	East Coast Bio HM1063	-	-	-	137
585	Sino Biological 40143-R001	East Coast Bio HM1064	-	-	-	91.5
586	Sino Biological 40143-R001	East Coast Bio HM1065	-	-	-	288.5
587	Sino Biological 40143-R001	East Coast Bio HM1066	-	-	-	185.5
588	Sino Biological 40143-R001	East Coast Bio HM1068	-	-	-	127
589	Sino Biological 40143-R001	East Coast Bio HM1069	-	-	-	182
590	Sino Biological 40143-R001	Fitzgerald 10-2856	-	17	-	-
591	Sino Biological 40143-R001	Fitzgerald 10-2857	-	48	-	-
592	Sino Biological 40143-R001	Fitzgerald 10-2860	-	-	-	307.5
593	Sino Biological 40143-R001	Fitzgerald 10-2861	-	-	120.5	-
594	Sino Biological 40143-R001	Genemedi GMP-V-2019nCoV-NAb001	-	-	53.5	-
595	Sino Biological 40143-R001	Genemedi GMP-V-2019nCoV-NAb002	-	-	48	-
596	Sino Biological 40143-R001	Medix Bio 100531	-	-	-	104
597	Sino Biological 40143-R001	Medix Bio 100532	-	-	-	229
598	Sino Biological 40143-R001	MyBiosource MBS569937	-	134.5	-	-
599	Sino Biological 40143-R001	MyBiosource MBS569939	-	118	-	-
600	Sino Biological 40143-R001	MyBiosource MBS569951	-	123.5	-	-
601	Sino Biological 40143-R001	MyBiosource MBS569961	-	-	86	-
602	Sino Biological 40143-R001	Novus Bio NB100-56683	76.5	-	-	-
603	Sino Biological 40143-R001	Sino Biological 40143-MM05	7.5	13.5	19	13
604	Sino Biological 40143-R001	Sino Biological 40143-MM08	4	17.5	25	15.5
605	Sino Biological 40143-R001	Sino Biological 40143-R001	90.5	-	-	-
606	Sino Biological 40143-R001	Sino Biological 40143-R004	11.5	29.5	68.5	-
607	Sino Biological 40143-R001	Sino Biological 40143-R019	82	-	-	-
608	Sino Biological 40143-R001	Sino Biological 40143-R040	100.5	-	-	-
609	Sino Biological 40143-R001	Sino Biological 40588-T62	43.5	-	-	-
610	Sino Biological 40143-R004	Bioss bsm-41411M	-	-	57.5	-
611	Sino Biological 40143-R004	Bioss bsm-41412M	-	-	87.5	-
612	Sino Biological 40143-R004	Bioss bsm-41413M	-	-	117.5	-

Table 1si (continued) | Antibody pairs screened in rounds 1-4 on LFAs.

Index	Capture antibody	Detector antibody	Average rank round 1	round 2	round 3	round 4	Index	Capture antibody	Detector antibody	Average rank round 1	round 2	round 3	round 4
613	Sino Biological 40143-R004	Bioss bsm-41414M	-	-	118.5	-	634	Sino Biological 40143-R040	Bioss bsm-41411M	-	24	20.5	-
614	Sino Biological 40143-R004	Creative Diagnostics CABT-RM320	80.5	-	-	-	635	Sino Biological 40143-R040	Bioss bsm-41412M	-	30.5	42	-
615	Sino Biological 40143-R004	Creative Diagnostics CABT-CS037	34.5	-	99	-	636	Sino Biological 40143-R040	Bioss bsm-41413M	-	-	64	-
616	Sino Biological 40143-R004	Creative Diagnostics DCABH-4693	45	-	-	-	637	Sino Biological 40143-R040	Bioss bsm-41414M	-	-	69	-
617	Sino Biological 40143-R004	Fitzgerald 10-2861	-	-	133.5	-	638	Sino Biological 40143-R040	Bioss bsm-41415M	-	45.5	-	-
618	Sino Biological 40143-R004	Genemedi GMP-V-2019nCoV-NAb001	-	-	141	-	639	Sino Biological 40143-R040	Creative Diagnostics CABT-RM320	105	147.5	-	-
619	Sino Biological 40143-R004	Genemedi GMP-V-2019nCoV-NAb002	-	-	102.5	-	640	Sino Biological 40143-R040	Creative Diagnostics CABT-CS037	14	34.5	13.5	-
620	Sino Biological 40143-R004	MyBiosource MBS569961	-	-	115	-	641	Sino Biological 40143-R040	Creative Diagnostics DCABH-4693	17	30.5	-	-
621	Sino Biological 40143-R004	Sino Biological 40143-MM05	49	-	78.5	-	642	Sino Biological 40143-R040	Fitzgerald 10-2856	-	29.5	-	-
622	Sino Biological 40143-R004	Sino Biological 40143-MM08	40	-	106.5	-	643	Sino Biological 40143-R040	Fitzgerald 10-2857	-	53	-	-
623	Sino Biological 40143-R004	Sino Biological 40143-R001	36.5	-	-	-	644	Sino Biological 40143-R040	Fitzgerald 10-2861	-	-	111	-
624	Sino Biological 40143-R004	Sino Biological 40143-R004	94.5	-	115	-	645	Sino Biological 40143-R040	Genemedi GMP-V-2019nCoV-NAb001	-	-	56.5	-
625	Sino Biological 40143-R004	Sino Biological 40143-R019	76	-	-	-	646	Sino Biological 40143-R040	Genemedi GMP-V-2019nCoV-NAb002	-	-	41	-
626	Sino Biological 40143-R004	Sino Biological 40143-R040	34.5	-	-	-	647	Sino Biological 40143-R040	MyBiosource MBS569937	-	79	-	-
627	Sino Biological 40143-R004	Sino Biological 40588-T62	40	-	-	-	648	Sino Biological 40143-R040	MyBiosource MBS569939	-	111	-	-
628	Sino Biological 40143-R019	Novus Bio NB100-56683	78	-	-	-	649	Sino Biological 40143-R040	MyBiosource MBS569951	-	132	-	-
629	Sino Biological 40143-R019	Sino Biological 40143-MM05	67.5	-	-	-	650	Sino Biological 40143-R040	MyBiosource MBS569961	-	144	96.5	-
630	Sino Biological 40143-R019	Sino Biological 40143-R001	94.5	-	-	-	651	Sino Biological 40143-R040	Novus Bio NB100-56683	87.5	-	-	-
631	Sino Biological 40143-R019	Sino Biological 40143-R019	101	-	-	-	652	Sino Biological 40143-R040	Sino Biological 40143-MM05	21.5	31	21.5	-
632	Sino Biological 40143-R019	Sino Biological 40143-R040	55	-	-	-	653	Sino Biological 40143-R040	Sino Biological 40143-MM08	11.5	3.5	16.5	-
633	Sino Biological 40143-R019	Sino Biological 40588-T62	66	-	-	-	654	Sino Biological 40143-R040	Sino Biological 40143-R001	68	-	-	-

Table 1si (continued) | Antibody pairs screened in rounds 1-4 on LFAs.

Index	Capture antibody	Detector antibody	Average rank				Index	Capture antibody	Detector antibody	Average rank			
			round 1	round 2	round 3	round 4				round 1	round 2	round 3	round 4
655	Sino Biological 40143-R040	Sino Biological 40143-R004	29	38.5	88.5	-	665	Sino Biological 40588-T62	MyBiosource MBS569961	-	103	-	-
656	Sino Biological 40143-R040	Sino Biological 40143-R019	74	-	-	-	666	Sino Biological 40588-T62	Novus Bio NB100-56683	100	-	-	-
657	Sino Biological 40143-R040	Sino Biological 40143-R040	73.5	-	-	-	667	Sino Biological 40588-T62	Sino Biological 40143-MM05	20.5	30	-	-
658	Sino Biological 40143-R040	Sino Biological 40588-T62	53.5	-	-	-	668	Sino Biological 40588-T62	Sino Biological 40143-MM08	11.5	29	-	-
659	Sino Biological 40588-T62	Creative Diagnostics CABT-RM320	75	81.5	-	-	669	Sino Biological 40588-T62	Sino Biological 40143-R001	36	-	-	-
660	Sino Biological 40588-T62	Creative Diagnostics CABT-CS037	20	37	-	-	670	Sino Biological 40588-T62	Sino Biological 40143-R004	23	36.5	-	-
661	Sino Biological 40588-T62	Creative Diagnostics DCABH-4693	17.5	34.5	-	-	671	Sino Biological 40588-T62	Sino Biological 40143-R019	56	-	-	-
662	Sino Biological 40588-T62	Fitzgerald 10-2857	-	64	-	-	672	Sino Biological 40588-T62	Sino Biological 40143-R040	59.5	-	-	-
663	Sino Biological 40588-T62	MyBiosource MBS569937	-	128.5	-	-	673	Sino Biological 40588-T62	Sino Biological 40588-T62	44.5	-	-	-
664	Sino Biological 40588-T62	MyBiosource MBS569939	-	84	-	-							

Table 2si | A list of anti-nucleocapsid antibodies and their commercial sources.

| Antibody Cat. No.	Vendor	Host	Isotype	Antibody Cat. No.	Vendor	Host	Isotype
Ab01690-10.0	Absolute Antibody	humanized	IgG1, kappa	GMP-V-2019nCov-NAb001	Genemedi	humanized	IgG1
Ab01691-10.0		humanized	IgG1, kappa	GMP-V-2019nCov-NAb002		humanized	sdFv-Fc
bsm-41411M		mouse	IgG2b	9547	Meridian Life Science	mouse	IgG1
bsm-41412M		mouse	IgG2b	9548		mouse	IgG1
bsm-41415M	Bioss Antibodies	mouse	IgG2b	MBS569951		mouse	mouse Mab
bsm-41413M		mouse	IgG2b	MBS569961		mouse	IgG
bsm-41414M		mouse	IgG2b	MBS569938		mouse	mouse Mab
CABT-RM320	Creative Diagnostics	rabbit	IgG	MBS569937	MyBiosource	mouse	mouse Mab
CABT-CS037		humanized	IgG	MBS569939		mouse	mouse Mab
DCABH-4693		mouse	IgG1	MBS569961		mouse	IgG
HM1066		mouse	IgG2a	NB100-56576		rabbit	IgG
HM1054		mouse	IgG2b	NB100-56683		rabbit	IgG
HM1055		mouse	IgG1	NB100-56049		rabbit	IgG
HM1056		mouse	IgG1	NB100-56576		polyclonal rabbit	IgG
HM1057		mouse	IgG1	NB100-56683		polyclonal rabbit	IgG
HM1058		mouse	IgG1	NB100-56049	Novus Biological	polyclonal rabbit	IgG
HM1063	EastCoast Bio	mouse	IgG1	NBP2-24747		monoclonal mouse	IgG2b, kappa
HM1064		mouse	-	NB100-56576		polyclonal rabbit	IgG
HM1065		mouse	-	NB100-56683		polyclonal rabbit	IgG
HM1068		mouse	IgG	NB100-56049		polyclonal rabbit	IgG
HM1069		mouse	IgG	40588-R0004		monoclonal rabbit	IgG
348717		mouse	IgG1	40143-MM08		monoclonal mouse	IgG1
349082		mouse	IgG1	40143-R001		monoclonal rabbit	IgG
10-CR9003M1		mouse	IgG2b	40143-R040	Sino Biological	monoclonal rabbit	IgG
10-CR9003M2		mouse	IgG1	40143-R004		monoclonal rabbit	IgG
10 2860	Fitzgerald	murine ascites	IgG	40588-T62		polyclonal rabbit	IgG
10 2861		murine ascites	IgG	40143-MM05		monoclonal mouse	IgG1
10 2856		murine ascites	IgG2b	40143-R019		monoclonal rabbit	IgG
10 2857		murine ascites	IgG1	PAB21469-250	The Native Antigen Co.	rabbit	IgG
348352		mouse	IgG1				
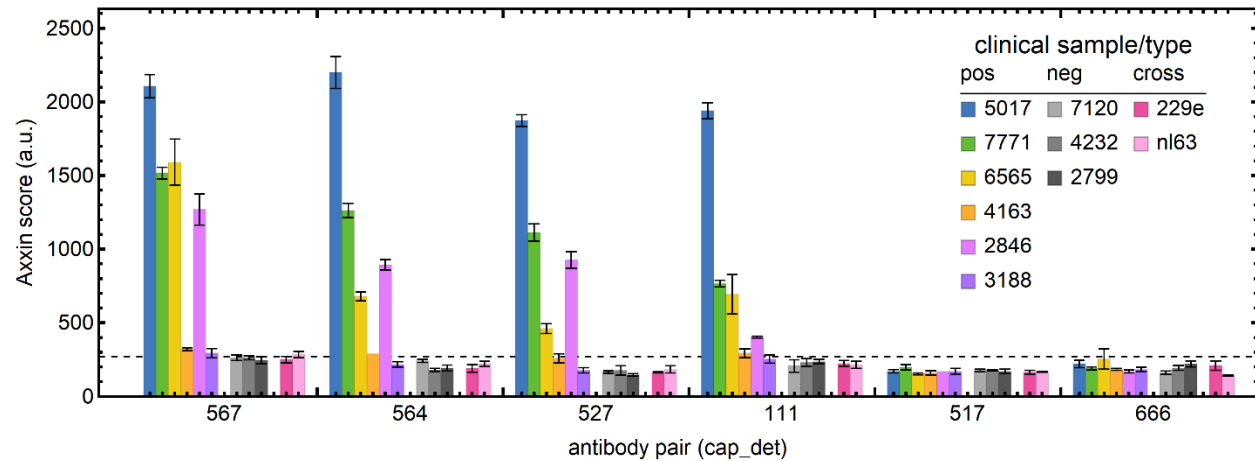
Figure 1si | Six antibody pairs were striped and capture and/or detectors in a lateral flow assay and screened with six RT-qPCR-confirmed SARS-CoV-2 banked clinical positive samples, three SARS-CoV-2-negative samples, and two potentially cross-reactive samples. Pairs 567 and 564 were chosen as relatively highly ranked pairs. Pairs 527 and 111 were chosen as middle ranked pairs. And, pairs 517 and 666 were chosen as low ranked pairs.
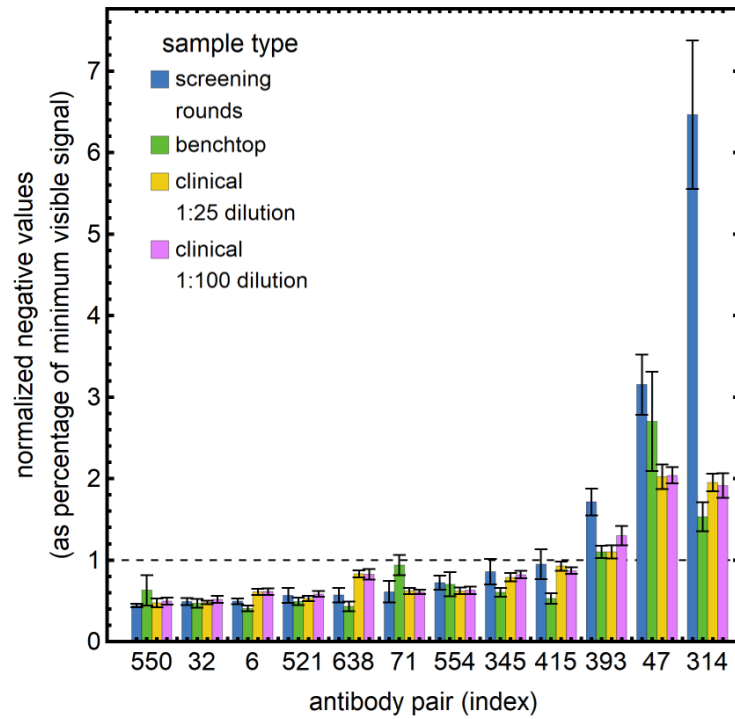
Figure 2si | Combining negative controls across several rounds of robotic screening data, benchtop tests with LFAs, and with diluted clinical negative samples shows that non-specific binding at the test line is predictable in the screening system. The black dotted line is the approximate threshold for test line visibility in an LFA.

Table 3si | Antibody pairs selected to be screened against clinical samples are ranked according to average performance by S-N and S/N in the clinical screen. Average rank from all four robot screening rounds are also shown.

Index	Capture antibody	Detector antibody	Average rank in clinical screen	Average rank rd. 1	rd. 2	rd. 3	rd. 4
567	Sino Biological 40143-MM08	Sino Biological 40143-R004	1	2.5	25	86.5	-
527	Sino Biological 40143-MM05	Sino Biological 40143-MM08	2	30	-	-	-
564	Sino Biological 40143-MM08	Sino Biological 40143-MM05	3	4	21.5	14	12
111	Creative Diagnostics CABT-CS037	Sino Biological 40143-R004	4.5	46.5	-	-	-
423	Genemedi GMP-V-2019nCoV-NAb001	Sino Biological 40143-MM08	5.5	-	-	8.5	2.5
7	Bioss bsm-41411M	Creative Diagnostics CABT-CS037	7.5	-	4	5.5	9
534	Sino Biological 40143-MM08	Bioss bsm-41412M	7.5	-	12.5	27	-
323	Fitzgerald 10-2856	Bioss bsm-41411M	8	-	43.5	32.5	44.5
46	Bioss bsm-41413M	Bioss bsm-41411M	9.5	-	-	36	23.5
355	Fitzgerald 10-2856	Sino Biological 40143-MM08	9.5	-	3.5	14	14
640	Sino Biological 40143-R040	Creative Diagnostics CABT-CS037	10.5	14	34.5	13.5	-
33	Bioss bsm-41411M	Sino Biological 40143-MM08	11	-	3	5.5	3
401	Genemedi GMP-V-2019nCoV-NAb001	Bioss bsm-41413M	11.5	-	-	23	-
517	Novus Bio NB100-56683	Sino Biological 40143-MM05	14.5	87	-	-	-
666	Sino Biological 40588-T62	Novus Bio NB100-56683	14.5	100	-	-	-
70	Bioss bsm-41413M	Sino Biological 40143-MM08	16	-	-	3	3.5