bioRxiv preprint doi: https://doi.org/10.1101/2020.05.21.109546. this version posted May 22, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.

A high-throughput neutralizing antibody assay for COVID-19 diagnosis and vaccine evaluation

Antonio E. Muruato[1,2#], Camila R. Fontes-Garfias[1#], Ping Ren[3#], Mariano A. Garcia-Blanco[1,4,5], Vineet D. Menachery[2,3,6], Xuping Xie[1*], Pei-Yong Shi[1,6,7,8*]

[1]Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston TX, USA

[2]Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston TX, USA

[3]Department of Pathology, University of Texas Medical Branch, Galveston TX, USA

[4]Programme in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore, Singapore

[5]Department of Internal Medicine, University of Texas Medical Branch, Galveston, TX, USA

[6]Institute for Human Infections and Immunity, University of Texas Medical Branch, Galveston, TX, USA

[7]Sealy Institute for Vaccine Sciences, University of Texas Medical Branch, Galveston, TX, USA

[8]Sealy Center for Structural Biology & Molecular Biophysics, University of Texas Medical Branch, Galveston, TX, USA

*C.F.-G., A.E.M., and P.R. contributed equally to this study

*Correspondence: X.X. (xuxie@UTMB.edu) or P.-Y.S. (peshi@UTMB.edu)

Running title: A high-throughput SARS-CoV-2 neutralizing assay

Keywords: SARS-CoV-2, COVID-19, SARS-CoV, diagnosis, vaccine, coronavirus

bioRxiv preprint doi: https://doi.org/10.1101/2020.05.21.109546. this version posted May 22, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.

1 **Abstract**

2 Virus neutralization remains the gold standard for determining antibody efficacy.
3 Therefore, a high-throughput assay to measure SARS-CoV-2 neutralizing antibodies is urgently
4 needed for COVID-19 serodiagnosis, convalescent plasma therapy, and vaccine development.
5 Here we report on a fluorescence-based SARS-CoV-2 neutralization assay that detects SARS-
6 CoV-2 neutralizing antibodies in COVID-19 patient specimens and yields comparable results to
7 plaque reduction neutralizing assay, the gold standard of serological testing. Our approach
8 offers a rapid platform that can be scaled to screen people for antibody protection from COVID-
9 19, a key parameter necessary to safely reopen local communities.

10

11 **Text**

12 The ongoing coronavirus disease 2019 (COVID-19) pandemic is caused by severe acute
13 respiratory syndrome coronavirus 2 (SARS-CoV-2), first reported in Wuhan, China in late
14 2019[1,2]. As of May 18, 2020, COVID-19 has caused 4.8 million confirmed infections and over
15 318,028 deaths worldwide (https://www.worldometers.info/coronavirus/). Many areas of the
16 world have been in lockdown mode to curb the viral transmission, but the reality is that COVID-
17 19 is here to stay until a safe and efficacious vaccine becomes available. The pandemic's
18 catastrophic economic impact is pushing governments to reopen their economies, and this
19 creates a public health quandary. At this time, our only option is to minimize viral transmission
20 through social distancing and contact tracing, which relies on the diagnosis of viral RNA through
21 RT-PCR (https://www.fda.gov/media/134922/download). Proper public health policy would be
22 greatly enhanced if we had a reliable and facile assay to measure the immune protection among
23 COVID-19 recovered patients.

24 Coronavirus infections typically induce neutralizing antibody responses[3]. The
25 seroconversion rates in COVID-19 patients are 50% and 100% on day 7 and 14 post symptom

bioRxiv preprint doi: https://doi.org/10.1101/2020.05.21.109546. this version posted May 22, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.

26 onset, respectively[4]. Given the unknown scale of asymptomatic infections, there is a pressing
27 need for serological diagnosis to determine the real number of infections. Such information is
28 essential for defining the case-fatality rate and for making the policy on the scale and duration of
29 social lockdowns. The serological assays are also required to identify donors with high-titers for
30 convalescent plasma for therapy, and to define correlates of protection from SARS-CoV-2.
31 While viral RNA-based testing for active infection is the current standard, surveying antibody
32 protection is a necessary part of any return to social normality.

33 For serodiagnosis, several COVID-19 assay platforms have achieved FDA emergency
34 use authorizations (EUA), including ELISA[5] (https://www.fda.gov/media/137029/download),
35 lateral flow immunoassay (https://www.fda.gov/media/136625/download), and Microsphere
36 Immunoassay (https://www.fda.gov/media/137541/download). These assays measure antibody
37 binding to SARS-CoV-2 spike protein. Since not all spike-binding antibodies can block viral
38 infection, these assay platforms do not functionally measure antibody inhibition of SARS-CoV-2
39 infection. An ideal serological assay should measure neutralizing antibody levels, which should
40 predict protection from reinfection. Conventionally, neutralizing antibodies are measured by
41 plaque reduction neutralization test (PRNT). Although PRNT and ELISA results generally
42 corelate with each other, the lack of complete fidelity of ELISA continues to make PRNT the
43 gold-standard for determining immune protection[6,7]. However, due to its low throughput, PRNT
44 is not practical for large scale serodiagnosis and vaccine evaluation. This is a major gap for
45 COVID-19 surveillance and vaccine development.

46 To address the above gap, we developed a fluorescence-based assay that rapidly and
47 reliably measures neutralization of a reporter SARS-CoV-2 by antibodies from patient
48 specimens. The assay was built on a stable mNeonGreen SARS-CoV-2 where the
49 mNeonGreen gene was engineered at the OFR7 of the viral genome[8]. Fig. 1a depicts the
50 flowchart of the reporter neutralization assay in a 96-well format. Briefly, patient sera were

bioRxiv preprint doi: https://doi.org/10.1101/2020.05.21.109546. this version posted May 22, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.

51 serially diluted and incubated with the reporter virus. After incubation at 37°C for 1 h, Vero E6
52 cells (pre-seeded in a 96-well plate) were infected with the virus/serum mixtures at a multiplicity
53 of infection (MOI) of 0.5. At 16 h post-infection, the mNeonGreen-positive cells were quantitated
54 using a high-content imaging reader (Fig. 1a). Forty COVID-19 serum specimens from RT-PCR-
55 confirmed patients and ten non-COVID-19 serum samples (archived before COVID-19
56 emergence) were analyzed using the reporter virus. After reporter viral infection, the cells turned
57 green in the absence of serum (Fig. 1b, bottom panel); in contrast, incubation of the reporter
58 virus with COVID-19 patient serum decreased the number of fluorescent cells (top panel). A
59 dose response curve was obtained between the number of fluorescent cells and the fold of
60 serum dilution (Fig. 1c), which allowed for determination of the dilution fold that neutralized 50%
61 of fluorescent cells (NT_{50}). The reporter assay rapidly diagnosed fifty specimens in less than 20
62 h: all forty COVID-19 sera (specimens 1-40) showed positive NT_{50} of 80 to 5152, and all ten
63 non-COVID-19 sera (specimens 41-50) showed negative NT_{50} of <20 for (Fig. 1d).

64 To validate the reporter virus neutralization results, we performed the conventional
65 PRNT on the same set of patient specimens. In agreement with the reporter virus results, the
66 forty positive sera showed $PRNT_{50}$ of 40 to 3200, and the ten negative sera exhibited $PRNT_{50}$ of
67 <20 (Fig. 1d). A strong correlation was observed between the reporter virus and PRNT results,
68 with a correlation efficiency R^2 of 0.9 (Fig. 1e). The results demonstrate that when diagnosing
69 patient specimens, the reporter virus assay delivers neutralization results comparable to the
70 PRNT assay, the gold standard of serological testing.

71 Next, we evaluated the specificity of reporter neutralization assay using potentially cross-
72 reactive sera and interfering substances (Table 1). Two groups of specimens were tested for
73 cross reactivity. Group I included 138 clinical sera from patients with antigens or antibodies
74 against different viruses, bacteria, and parasites. Group II consisted of 19 samples with albumin,
75 elevated bilirubin, cholesterol, rheumatoid factor, and autoimmune nuclear antibodies. None of

bioRxiv preprint doi: https://doi.org/10.1101/2020.05.21.109546. this version posted May 22, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.

76 the specimens cross neutralized mNeonGreen SARS-CoV-2 (Table 1), including the four
77 common cold coronaviruses (NL63, 229E, OC43, and HUK1). The latter result is consistent with
78 the recent reports that sera from common cold coronavirus patients did not cross react with
79 SARS-CoV-2[5,9]. However, more specimens are required to further validate the cross reactivity,
80 particularly between SARS-CoV-2 and other human coronaviruses, including SARS-CoV-1 and
81 MERS-CoV.

82 In this study, we developed a rapid fluorescence-based high-throughput assay for
83 COVID-19 serodiagnosis. The reporter virus assay is superior to antigen/antibody binding
84 assays because it measures functional SARS-CoV-2 neutralizing activity in the specimens.
85 When diagnosing patient sera, the reporter virus assay generated NT_{50} values comparable to
86 the conventional PRNT assay. Compared with the PRNT assay, our reporter neutralization test
87 has shortened the assay turnaround time by several days and increased the testing capacity to
88 high throughput. Previously, lentiviruses or vesicular stomatitis virus (VSV) pseudotyped with
89 SARS-CoV-2 spike protein have been reported for neutralization assays[10]. One weakness of the
90 spike pseudotyped assay is that it lacks the same composition of an actual virion, including the
91 SARS-CoV-2 M or E proteins. In addition, the spike protein conformation, either the trimer or
92 monomer, may be different in the pseudotypted virus as compared with the authentic SARS-
93 CoV-2 virion.

94 Since mNeonGreen SARS-CoV-2 is stable and replicates like wild-type virus, our
95 reporter neutralization assay provides an ideal model for high-throughput serological testing. As
96 the mNeonGreen SARS-CoV-2 grows to >10^7 PFU/ml in cell culture[8], the reporter virus can be
97 easily scaled up for testing large sample volumes. Besides mNeonGreen, we have begun to
98 develop other reporter SARS-CoV-2 (*e.g.*, luciferase or mCherry) that can also be used for such
99 serological testing. Although the current study performed the assay in a 96-well format, the
100 assay can be readily adapted to 384- and 1536-well formats. Despite the strengths of high

bioRxiv preprint doi: https://doi.org/10.1101/2020.05.21.109546. this version posted May 22, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.

101 throughput and reliability, the current reporter neutralization assay must be performed in
102 biosafety level 3 (BSL3) containment. Efforts are ongoing to engineer an attenuated version of
103 SARS-CoV-2 so that the assay could be performed at a BSL2 facility. Nevertheless, the
104 mNeonGreen reporter assay offers a rapid, high-throughput platform to test COVID-19 patient
105 sera not previously available.

106 Because neutralizing titer is a key parameter to predict immunity, the reporter
107 neutralization assay should be useful for high-throughput evaluation of COVID-19 vaccines and
108 for identification of high neutralizing convalescent plasma for therapy. Indeed, treatment of
109 severe COVID-19 patients with convalescent plasma shows clinical benefits[11]. For vaccine
110 development, a standardized neutralizing assay will facilitate down selection of various
111 candidates for clinical development. Furthermore, the reporter assay could be used over time to
112 monitor the waning of protective neutralizing titers in COVID-19 patients and to study the
113 correlates of protection from SARS-CoV-2. Thus, the ability to rapidly measure neutralizing
114 antibody levels in populations is essential for guiding policymakers to reopen the economy and
115 society, deploy healthcare workers, and prepare for SARS-CoV-2 reemergence.

116

117 **References**

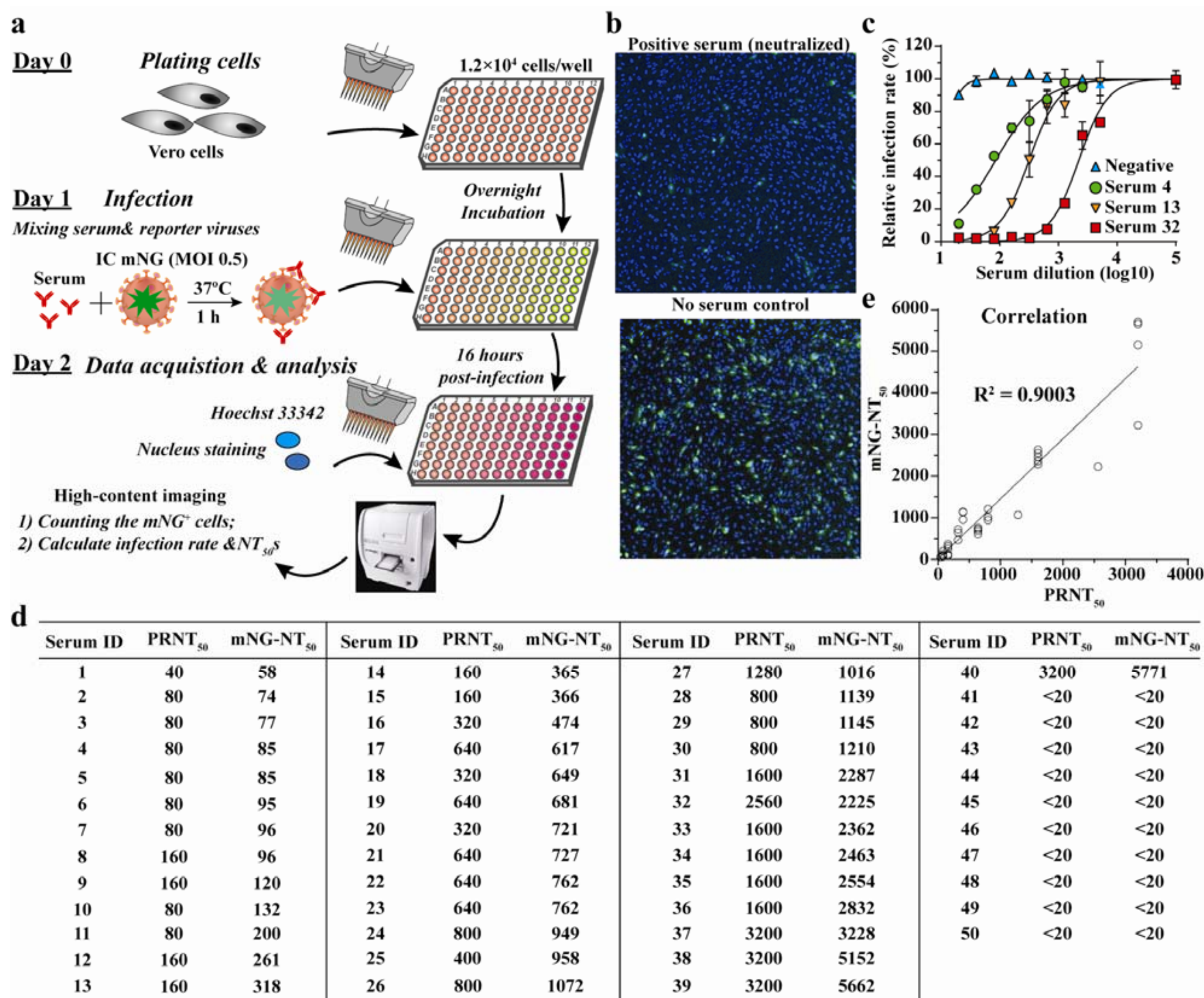
118

119 1 Zhou, P. *et al.* A pneumonia outbreak associated with a new coronavirus of probable bat
120 origin. *Nature* **579**, 270-273, doi:10.1038/s41586-020-2012-7 (2020).
121 2 Zhu, N. *et al.* A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl*
122 *J Med* **382**, 727-733, doi:10.1056/NEJMoa2001017 (2020).
123 3 Huang, A. T. *et al.* A systematic review of antibody mediated immunity to coronaviruses:
124 antibody kinetics, correlates of protection, and association of antibody responses with
125 severity of disease. *medRiv* (2020).
126 4 Wolfel, R. *et al.* Virological assessment of hospitalized patients with COVID-2019.
127 *Nature*, doi:10.1038/s41586-020-2196-x (2020).
128 5 Amanat, F. *et al.* A serological assay to detect SARS-CoV-2 seroconversion in humans.
129 *Nat Med*, doi:10.1038/s41591-020-0913-5 (2020).
130 6 Perera, R. A. *et al.* Serological assays for severe acute respiratory syndrome
131 coronavirus 2 (SARS-CoV-2), March 2020. *Euro Surveill* **25**, doi:10.2807/1560-
132 7917.ES.2020.25.16.2000421 (2020).

bioRxiv preprint doi: https://doi.org/10.1101/2020.05.21.109546. this version posted May 22, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.

133 7 Okba, N. M. A. *et al.* Severe Acute Respiratory Syndrome Coronavirus 2-Specific
134 Antibody Responses in Coronavirus Disease 2019 Patients. *Emerg Infect Dis* **26**,
135 doi:10.3201/eid2607.200841 (2020).
136 8 Xie, X. *et al.* An infectious cDNA clone of SARS-CoV-2. *Cell Host Microbe*,
137 doi:10.1016/j.chom.2020.04.004 (2020).
138 9 Khan, S. *et al.* Analysis of Serologic Cross-Reactivity Between Common Human
139 Coronaviruses and SARS-CoV-2 Using Coronavirus Antigen Microarray. *bioRxiv* (2020).
140 10 Nie, J. *et al.* Establishment and validation of a pseudovirus neutralization assay for
141 SARS-CoV-2. *Emerg Microbes Infect* **9**, 680-686, doi:10.1080/22221751.2020.1743767
142 (2020).
143 11 Shen, C. *et al.* Treatment of 5 Critically Ill Patients With COVID-19 With Convalescent
144 Plasma. *JAMA*, doi:10.1001/jama.2020.4783 (2020).
145 12 Shan, C. *et al.* Evaluation of a Novel Reporter Virus Neutralization Test for Serological
146 Diagnosis of Zika and Dengue Virus Infection. *J Clin Microbiol* **55**, 3028-3036,
147 doi:10.1128/JCM.00975-17 (2017).
148 13 Shan, C. *et al.* A Rapid Zika Diagnostic Assay to Measure Neutralizing Antibodies in
149 Patients. *EBioMedicine* **17**, 157-162, doi:10.1016/j.ebiom.2017.03.006 (2017).
150

bioRxiv preprint doi: https://doi.org/10.1101/2020.05.21.109546. this version posted May 22, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.



Serum ID	$PRNT_{50}$	$mNG\text{-}NT_{50}$	Serum ID	$PRNT_{50}$	$mNG\text{-}NT_{50}$	Serum ID	$PRNT_{50}$	$mNG\text{-}NT_{50}$	Serum ID	$PRNT_{50}$	$mNG\text{-}NT_{50}$
1	40	58	14	160	365	27	1280	1016	40	3200	5771
2	80	74	15	160	366	28	800	1139	41	<20	<20
3	80	77	16	320	474	29	800	1145	42	<20	<20
4	80	85	17	640	617	30	800	1210	43	<20	<20
5	80	85	18	320	649	31	1600	2287	44	<20	<20
6	80	95	19	640	681	32	2560	2225	45	<20	<20
7	80	96	20	320	721	33	1600	2362	46	<20	<20
8	160	96	21	640	727	34	1600	2463	47	<20	<20
9	160	120	22	640	762	35	1600	2554	48	<20	<20
10	80	132	23	640	762	36	1600	2832	49	<20	<20
11	80	200	24	800	949	37	3200	3228	50	<20	<20
12	160	261	25	400	958	38	3200	5152			
13	160	318	26	800	1072	39	3200	5662			

Figure 1. A high-throughput neutralizing antibody assay for COVID-19 diagnosis. (a) Assay flowchart. mNeonGreen SARS-CoV-2 was neutralized with COVID-19 patient sera. Vero E6 cells were infected with the reporter virus/serum mixture with an MOI of 0.5. The fluorescence of infected cells was quantified to estimate the NT_{50} value for each serum. (b) Representative images of reporter virus-infected Vero E6 cells. Images for a positive neutralizing serum (top panel) and no serum control (bottom panel) are presented. (c) Neutralization curves. Representative neutralization curves are presented for three positive sera and one negative sera. (d) Summary of NT_{50} values of fifty patient sera. The NT_{50} values from both reporter virus and conventional PRNT assays are presented. (e) Correlation analysis of

bioRxiv preprint doi: https://doi.org/10.1101/2020.05.21.109546. this version posted May 22, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.

161 NT_{50} values between the reporter virus and PRNT assays. The correlation efficiency R^2 is
162 indicated.

bioRxiv preprint doi: https://doi.org/10.1101/2020.05.21.109546. this version posted May 22, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.

163 **Table 1. Cross reactivity of mNeonGreen SARS-CoV-2 neutralization assay**
164

*Immune sera and #interfering substances	Sample number	Cross reactivity
Anti-Chikungunya virus	4	0
Cryptococcus neoformans antigen	2	0
Anti-Cytomegalovirus	8	0
Anti-Dengue virus	5	0
Anti-Epstein Barr Virus: capsid or nuclear antigen	8	0
Anti-Hepatitis A virus	5	0
Anti-Hepatitis B virus: surface antigen	14	0
Anti-Hepatitis C virus	3	0
Anti-Herpes simplex virus 1	7	0
Anti-Herpes simplex virus 2	5	0
Human coronavirus 229E	1	0
Human coronavirus HKU1	3	0
Human coronavirus NL63	1	0
Human coronavirus OC43	4	0
Anti-Human immunodeficiency virus 1	7	0
Human rhinovirus	3	0
Influenza B virus	2	0
Anti-Measles virus	7	0
Anti-Mumps virus	5	0
Parainfluenza virus 2	1	0
Parainfluenza virus 4	1	0
Anti-Parvovirus B19	4	0
Anti-Rubella virus	9	0
Anti-Syphilis	4	0
Anti-Toxoplasma	2	0
Anti-Typhus Fever	1	0
Varicella zoster virus	13	0
West Nile Virus	3	0
Anti-Yellow fever virus: vaccination	2	0
Anti-Zika virus	4	0
#Albumin (4.5 g/dL)	3	0
#Elevated bilirubin conjugated (>0.4 mg/dL)	3	0
#Elevated bilirubin unconjugated (>0.8 mg/dL)	3	0
#Elevated cholesterol (>200 mg/dL)	3	0
#Elevated rheumatoid factor (>100 IU/mL)	3	0
#Nuclear antibodies	4	0

165
166 *A total of 138 sera with antigens or antibodies against different infections (or immunizations)
167 were tested against mNeonGreen SARS-CoV-2 neutralization assay. The immune sera are
168 listed in alphabetical order.
169 #Tested interfering substances and autoimmune disease nuclear antibodies.

bioRxiv preprint doi: https://doi.org/10.1101/2020.05.21.109546. this version posted May 22, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.

170 **Methods**

171 **mNeonGreen SARS-CoV-2.** The virus stock of mNeonGreen SARS-CoV-2 was
172 produced using an infectious cDNA clone of SARS-CoV-2 in which the ORF7 of the viral
173 genome was replaced with reporter mNeonGreen gene[8]. After rescued from the genome-length
174 viral RNA-electroporated cells, the viral stock was prepared by amplifying the mNeonGreen
175 SARS-CoV-2 on Vero E6 cells for one or two rounds. The titer of the virus stock was determined
176 by a standard plaque assay.

177 **Human sera and interfering substances.** All suman serum specimens were obtained
178 at the University of Texas Medical Branch (UTMB). All specimens were de-identified from
179 patient information. A total of forty de-identified convalescent sera from COVID-19 patients
180 (confirmed with viral RT-PCR positive) were tested in this study. Ten non-COVID-19 sera,
181 collected before COVID-19 emergence[12,13], were also tested in the reporter virus and PRNT
182 assays. For testing cross reactivity, a total of 138 de-identified specimens from patients with
183 antigens or antibodies against different viruses, bacteria, and parasites were tested in the
184 mNeonGreen SARS-COV-2 neutralization assay (Table 1). For testing interfering substances,
185 nineteen de-identified serum specimens with albumin, elevated bilirubin, cholesterol, rheumatoid
186 factor, and autoimmune nuclear antibodies were tested in the reporter neutralization assay. All
187 human sera were heat-inactivated at 56°C for 30 min before testing.

188 **mNeonGreen SARS-CoV-2 reporter neutralization assay.** Vero E6 cells (1.2×10^4) in
189 50 µl of DMEM (Gibco) containing 2% FBS (Hyclone) and 100 U/ml Penicillium-Streptomycin
190 (P/S; Gibco) were seeded in each well of black µCLEAR flat-bottom 96-well plate (Greiner Bio-
191 one™). The cells were incubated overnight at 37°C with 5% CO_2. On the following day, each
192 serum was 2-fold serially diluted in 2% FBS and 100 U/ml P/S DMEM, and incubated with
193 mNeonGreen SARS-CoV-2 at 37°C for 1 h. The virus-serum mixture was transferred to the
194 Vero E6 cell plate with the final multiplicity of infection (MOI) of 0.5. For each serum, the starting
195 dilution was 1/20 with nine 2-fold dilutions to the final dilution of 1/5120. After incubating the

bioRxiv preprint doi: https://doi.org/10.1101/2020.05.21.109546. this version posted May 22, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.

196 infected cells at 37°C for 16 h, 25 µl of Hoechst 33342 Solution (400-fold diluted in Hank's
197 Balanced Salt Solution; Gibco) were added to each well to stain cell nucleus. The plate was
198 sealed with Breath-Easy sealing membrane (Diversified Biotech), incubated at 37°C for 20 min,
199 and quantified for mNeonGreen fluorescence on Cytation™ 7 (BioTek). The raw images (2×2
200 montage) were acquired using 4× objective, processed, and stitched using the default setting.
201 The total cells (indicated by nucleus staining) and mNeonGreen-positive cells were quantified
202 for each well. Infection rates were determined by dividing the mNeonGreen-positive cell number
203 to total cell number. Relative infection rates were obtained by normalizing the infection rates of
204 serum-treated groups to those of non-serum-treated controls. The curves of the relative
205 infection rates versus the serum dilutions (log10 values) were plotted using Prism 8 (GraphPad).
206 A nonlinear regression method was used to determine the dilution fold that neutralized 50% of
207 mNeonGreen fluorescence (NT_{50}). Each serum was tested in duplicates. All mNeonGreen
208 SARS-CoV-2 reporter neutralization assay was performed at the BSL-3 facility at UTMB.

209 **Plaque reduction neutralization test (PRNT)**. Vero E6 cells (1.2×10^6 per well) were
210 seeded to 6-well plates. On the following day, 100 PFU of infectious clone-derived wild-type
211 SARS-CoV-2 was incubated with serially diluted serum (total volume of 200 µl) at 37°C for 1 h.
212 The virus-serum mixture was added to the pre-seeded Vero E6 cells. After 1 h 37°C incubation,
213 2 ml of 2% high gel temperature agar (SeaKem) in DMEM containing 5% FBS and 1% P/S was
214 added to the infected cells. After 2 days of incubation, 2 ml neutral red (1 g/l in PBS; Sigma)
215 was added to the agar-covered cells. After another 5-h incubation, neutral red was removed.
216 Plaques were counted for NT_{50} calculation. Each serum was tested in duplicates. The PRNT
217 assay was performed at the BSL-3 facility at UTMB.

218 **Statistical analysis.** The correlation of the NT_{50} values from mNeonGreen reporter
219 SARS-CoV-2 assay and the $PRNT_{50}$ values from plaque neutralization assay was analyzed
220 using a linear regression model in the software Prism 8 (GraphPad).

bioRxiv preprint doi: https://doi.org/10.1101/2020.05.21.109546. this version posted May 22, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.

221

222 **Data availability**

223 The results presented in the study are available upon request from the corresponding
224 authors. The mNeonGreen reporter SARS-CoV-2 has been deposited to the World Reference
225 Center for Emerging Viruses and Arboviruses (https://www.utmb.edu/wrceva) at UTMB for
226 distribution.

227

228 **Acknowledgements**

229 We thank colleagues at UTMB for helpful discussion during the course of this project.
230 P.-Y.S. was supported by NIH grants AI142759, AI134907, AI145617, and UL1TR001439, and
231 awards from the Sealy & Smith Foundation, Kleberg Foundation, John S. Dunn Foundation,
232 Amon G. Carter Foundation, Gilson Longenbaugh Foundation, and Summerfield Robert
233 Foundation. M.A.G.-B. was supported by NIH grant CA204806 and the Vacek Distinguished
234 Chair. V.D.M. was supported by NIH grants U19AI100625, R00AG049092, R24AI120942, and
235 STARs Award from the University of Texas System. A.E.M. is supported by a Clinical and
236 Translational Science Award NRSA (TL1) Training Core (TL1TR001440) from NIH. C.R.F.-G. is
237 supported by the predoctoral fellowship from the McLaughlin Fellowship Endowment at
238 UTMB.

239

240 **Author contributions**

241 P.R., M.A.G.-B., V.D.M., X.X., and P.-Y.S conceived the study. A.E.M. and C.R.F.-G.
242 performed the experiments and analyzed the results. P.R. prepared the serum specimens.
243 M.A.G.-B., V.D.M., X.X., and P.-Y.S wrote the manuscript.

244

bioRxiv preprint doi: https://doi.org/10.1101/2020.05.21.109546. this version posted May 22, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.

245 **Competing interests**

246 UTMB has filed a patent on the reverse genetic system and reporter SARS-CoV-2.