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SARS-CoV-2 seroprevalence and neutralizing activity in donor and patient blood from the San Francisco Bay Area.

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1   **SARS-CoV-2 seroprevalence and neutralizing activity in donor and patient blood from the**

2   **San Francisco Bay Area**

3

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43    **ABSTRACT**

44    We report very low SARS-CoV-2 seroprevalence in two San Francisco Bay Area populations.

45    Seropositivity was 0.26% in 387 hospitalized patients admitted for non-respiratory indications

46    and 0.1% in 1,000 blood donors. We additionally describe the longitudinal dynamics of

47    immunoglobulin-G, immunoglobulin-M, and *in vitro* neutralizing antibody titers in COVID-19

48    patients. Neutralizing antibodies rise in tandem with immunoglobulin levels following symptom

49    onset, exhibiting median time to seroconversion within one day of each other, and there is >93%

50    positive percent agreement between detection of immunoglobulin-G and neutralizing titers.

51

52    Coronavirus disease 2019 (COVID-19) is a novel respiratory illness caused by the severe acute

53    respiratory syndrome coronavirus 2 (SARS-CoV-2)[1]. The symptoms of COVID-19 range from

54    asymptomatic infection to acute respiratory distress syndrome and death, and the COVID-19

55    pandemic has resulted in substantial burdens on healthcare systems worldwide[2,3]. Given the

56    current state of diagnostic testing which largely relies on molecular techniques, the

57    seroprevalence of SARS-CoV-2-specific antibodies in different populations remains unclear.

58    Accurate and large-scale serologic testing that includes detection of neutralizing antibodies is

59    essential in evaluating spread of infection in the community, informing public health

60    containment efforts, and identifying donors for convalescent plasma therapy trials.

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**Performance Characteristics of the Abbott Architect IgG and IgM SARS-CoV-2 Assays**

We first assessed the performance of the Abbott Architect SARS-CoV-2 IgG (FDA Emergency Use Authorization (EUA)) and IgM (prototype) assays from a cohort of five outpatients and 38 hospitalized patients at University of California, San Francisco (UCSF) Medical Center and the San Francisco Veterans Affairs (SFVA) Health Care System. These assays are chemiluminescent microparticle immunoassays that target the nucleocapsid and spike proteins, respectively. All patients received care at adult inpatient units or clinics and were RT-PCR positive for SARS-CoV-2 from nasopharyngeal and/or oropharyngeal swab testing (**Figure 1A, Table S1**). The percentage of patients seroconverting for IgG at weekly time intervals following reported symptom onset reached 94.4% at ≥22 days (**Figure 1B**). Correspondingly, IgG assay sensitivity from analysis of all 423 samples increased weekly to reach 96.9% at ≥22 days, and was 99% when samples from seven immunocompromised patients (see below) were excluded (**Figure 1D, Table 1**). The percentage of patients seroconverting for IgM was also 94.4% at ≥22 days (**Figure 1E**) and IgM assay sensitivity from analysis of 346 samples was 97.9% (98.9% with immunocompromised patients excluded) (**Figure 1G, Table 1**).

Of the four patients who had not seroconverted for IgG by the end of 14 days (**Figure 1B**), two were kidney transplant recipients on tacrolimus and mycophenolate mofetil (MMF) immunosuppressive therapy; one was >90 years old; and one was an asymptomatic patient receiving acute psychiatric care who provided an unreliable history. Both renal transplant recipients were observed to ultimately seroconvert for IgG and IgM. Notably, delayed seroconversion for IgG and IgM was not universal in immunosuppressed patients: three additional solid organ transplant (SOT) recipients on tacrolimus and MMF, as well as one patient with rheumatoid arthritis on methotrexate and infliximab, all seroconverted within two weeks. A further SOT recipient was positive for IgG and IgM in the earliest available serum sample from day 17 post symptom onset (**Figure 2D, E**). We did not have samples beyond day 18 for the remaining two patients. However, as seroconversion was observed as late as three weeks after symptom onset (**Figure 2D, E**), it is possible that analysis of later samples would have demonstrated detectable antibodies in their serum. The one patient who was still IgG negative in the 22+ day time frame (**Figure 1B**) (from a plasma sample collected on day 29) had only mild symptoms and was positive by IgM and neutralizing antibody testing (described below). Conversely, the one patient who was IgM negative in the 22+ day time frame was both IgG and

94    neutralizing antibody positive from a plasma sample collected on day 50 (**Figure 1E**), by whichh

95    time IgM antibody titers may have waned significantly.

96         To evaluate assay specificity, serum and plasma samples collected by Abbott

97    Laboratories from US blood donors prior to the COVID-19 pandemic (pre-COVID-19) were

98    tested for IgG (n=1,013) and IgM (n=1,492) seroreactivity. Two samples out of 1,013 were

99    positive by IgG testing, yielding a specificity of 99.8% (95% CI: 99.3-100%) (**Figure 1C**),

100   concordant with the 99.9% specificity reported in a study by the University of Washington[4,5].

101   Similarly, testing of 235 remnant plasma samples from 163 SARS-CoV-2 PCR-negative UCSF

102   patients collected from late March to early April 2020 resulted in detection of only one positive

103   sample, yielding a specificity of 99.6% (95% CI: 97.7-100%) (**Figure 1H**). The IgG positive

104   sample was from a patient admitted for syncope but who reported a cough of one-month

105   duration, suggesting a potential prior infection with SARS-CoV-2. Six samples out of 1,492 from

106   US blood donors were positive by IgM testing, yielding a specificity of 99.6% (95% CI: 99.2-

107   99.9%) (**Figure 1F**). This was consistent with more limited testing of 39 SARS-CoV-2 PCR

108   negative UCSF patients, none of whom were positive for IgM antibody (**Figure 1I**). Thus, the

109   Architect SARS-CoV-2 IgG and IgM assays demonstrated high sensitivity (96.9%-97.9% at ≥22

110   days in a primarily hospitalized patient cohort) and specificity (99.6-99.8% in pre-COVID blood

111   donors), with good correlation (*rho* = 0.65) between IgG anti-nucleocapsid protein and IgM anti-

112   spike protein seropositivity (**Figure 2A**).

113

**Seroprevalence of SARS-CoV-2 in blood donors and patients from the San Francisco Bay**

**Area in March 2020**

116        Next, to investigate SARS-CoV-2 seroprevalence in the San Francisco Bay Area, we

117   collected plasma and serum samples from two cohorts of individuals with low suspicion of

118   infection from COVID-19. One cohort consisted of 1,000 individuals who donated blood in

119   March 2020 at blood bank centers throughout the Bay Area **(Figure 1A, Table S2).** Routine

120   blood donor screening was performed to exclude those with self-reported symptoms of acute

121   illness and abnormal vital signs. We detected four IgG positive samples in this cohort, yielding a

122   seroreactivity rate of 0.40% (**Figure 1H).** This cohort was not tested for IgM antibody. We then

123   analyzed the four samples using two orthogonal tests, the VITROS anti-SARS-CoV-2 total

124   antibody assay (Ortho Clinical Diagnostics EUA) and a SARS-CoV-2 pseudovirus neutralization

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125  assay (described below). Three of four samples were negative by both the VITROS and

126  neutralization assays, and thus were designated likely false positives by the Architect IgG assay.

127  Thus, the calculated seroprevalence after confirmatory orthogonal testing for Bay Area blood

128  donors in March 2020 was 0.1% (95% CI: 0.00% - 0.56%). The false positive rate in this

129  population of 0.3% is consistent with with the reported specificity of the Architect SARS-CoV-

130  IgG test of 99.6%[4].

131  The other cohort for evaluating seroprevalence represented a cross-section of patients

132  who received care at adult inpatient units or clinics at the UCSF Medical Center for indications

133  other than COVID-19 respiratory disease (non-COVID-19, never tested for SARS-CoV-2 by

134  RT-PCR) from late March to early April 2020. Remnant samples from 532 blood draws taken

135  from these 387 patients were obtained from UCSF clinical laboratories. Of these 532 samples,

136  five were positive for IgG; strikingly, all five of these samples were from the same patient who

137  had respiratory failure and ground-glass opacities on chest imaging but was never tested for

138  SARS-2-CoV by RT-PCR (**Figure 1H**). IgG seroprevalence in this population was thus 0.26%

139  (95% CI: 0-0.76%). Although only 23 of the 532 remnant samples were able to be subsequently

140  tested for IgM antibodies, importantly, none were positive (**Figure 1I**).

141

142

143  **Longitudinal dynamics of immunoglobulin and neutralizing antibody titers in SARS-CoV-**

144  **2 infected patient**

145  We next analyzed the longitudinal dynamics of plasma IgG (286 samples) and IgM (249

146  samples) levels in our cohort of 43 patients who were positive for SARS-CoV-2 by PCR.  As

147  previously reported, IgG and IgM antibody levels were observed to rise approximately in tandem

148  (**Figure 2D, E**)[6–10]. We correlated median IgG, IgM, and neutralizing antibody (described below)

149  levels at the weekly time intervals with severity of disease, and the differences were not

150  statistically significant.

151  Lastly, we sought to correlate IgG and IgM seropositivity with SARS-CoV-2 *in vitro*

152  neutralizing activity against a SARS-CoV-2 pseudovirus (a vesicular stomatitis virus (VSV)

153  pseudotype expressing the SARS-CoV-2 spike protein). Plasma titers that achieved 80%

154  neutralization of pseudovirus infectivity (NT80) were measured by luciferase assay (see

155  Methods). We compared NT80 with IgG and IgM measurements in 54 available plasma samples

156  from 22 of the 43 SARS-CoV-2 PCR positive patients (**Figure 2B, C**). The positive percent

157  agreement (PPA) between NT80 and IgG positivity was 93.8% and the negative percent

158  agreement (NPA) was 75.0% (**Figure 2C**). Results from the NT80 and IgM comparison were

159  similar, with a PPA of 84.8% and NPA of 78.6% (**Figure 2B**). Importantly, neutralizing titers

160  appeared concomitantly in plasma with IgG and IgM positivity (**Figure 2D-G**), correlated well

161  with IgG (*rho* = 0.79) and IgM (*rho* = 0.77) levels, and increased over time in parallel with the

162  rise of anti-spike IgM and ant-nucleocapsid IgG antibodies (**Figure 2H-I**).

163

164  **Conclusions**

165      In this study, we provide evidence that seropositive results using the Architect SARS-

166  CoV-2 anti-nucleocapsid protein IgG and anti-spike IgM assays are generally predictive of *in*

167  *vitro* neutralizing capacity. This correlation may have particular relevance for recovered COVID-

168  19 patients and the identification of candidate donors to provide blood for convalescent plasma

169  therapy. However, *in vitro* neutralization activity may not confer protective immunity and the

170  efficacy of convalescent plasma therapy for treatment of COVID-19 disease remains to be

171  determined. Our results also show that the seroprevalence of IgG antibodies against SARS-CoV-

172  2 in blood donors and non-COVID-19 patients seen at a tertiary care hospital in the San

173  Francisco Bay Area from March to April 2020 is very low at 0.10% (95% CI: 0.00% - 0.56%).

174  and 0.26% (0.00% - 0.76%), respectively. These seroprevalence rates in two distinct populations

175  in the San Francisco Bay Area are near the specificity limit of the Architect assay, and are far

176  lower than the specificity limits for many lateral flow immunoassays[11]. Our findings contrast

177  with those from other community-based studies that reported higher rates of seropositivity in

178  California[12,13], and underscore the importance of using a highly accurate test for surveillance

179  studies in low-prevalence populations. They also indicate a very low likelihood of widespread

180  cryptic circulation of SARS-CoV-2 in the Bay Area prior to March 2020, consistent with the low

181  detection rate by direct viral testing of respiratory samples collected during that early time

182  period[14].

183

184  **METHODS**

185  **Study design and Ethics**

186  The study population consisted of patients with available remnant serum and plasma specimens
187  from the clinical laboratories at University of California, San Francisco (UCSF). Samples from
188  patients who were positive or negative by SARS-CoV-2 real-time polymerase chain reaction
189  (RT-PCR) testing of nasopharyngeal, oropharyngeal, and/or pooled nasopharyngeal-
190  oropharyngeal swabs were collected in March – April 2020. Additional samples were collected
191  from randomly selected cohorts of outpatients and hospitalized patients at UCSF during the same
192  time period seen for indications other than COVID-19 respiratory disease (non-COVID). Serum
193  samples from blood donors in the San Francisco Bay Area were collected by Vitalant Research
194  Institute in March 2020. Clinical data for UCSF patients were extracted from electronic health
195  records and entered in a HIPAA (Health Insurance Portability and Accountability Act)-secure
196  REDCap research database. Collected data included demographics, major comorbidities, patient-
197  reported symptom onset date, clinical symptoms and indicators of COVID-19 severity such as
198  admission to the intensive care unit and requirement for mechanical ventilation. This study was
199  approved by the institutional review board (IRB) at UCSF (UCSF IRB #10-02598) as a no-
200  subject contact study with waiver of consent.

201

202  **Serologic testing**

203  The Abbott Architect SARS-CoV-2 IgG assay (FDA Emergency Use Authorization (EUA)) and
204  SARS-CoV-2 IgM (prototype) testing was performed using either serum or plasma samples on
205  the Architect instrument according to the manufacturer instructions[4]. These tests are
206  chemiluminescent microparticle immunoassay reactions that target the nucleocapsid protein (IgG
207  assay) or the spike protein (IgM assay) and measure relative light units that are then used to
208  calculate an index value. At a predefined index value threshold of 0.6 signal-to-cutoff (S/C) ratio
209  for IgM seropositivity and 1.4 S/C for IgG for seropositivity, these assays were found to have
210  specificities of 99.6% - 99.8%.

211

212  The VITROS anti-SARS-CoV-2 total antibody assay approved under FDA Emergency Use
213  Authorization was performed using either serum or plasma samples at Vitalant Research Institute
214  according to the manufacturer instructions[15]. The test is a chemiluminescent immunoassay that
215  targets the spike protein and measures relative light units that are then used to calculate an index
216  value. At a predefined index value threshold of 1.0 signal-to-cutoff (S/C) ratio for IgG

7

217   seropositivity, this assay was found to have a sensitivity of 100% (92.7% - 100%) and specificity

218   of 100% (95% CI = 99.1% - 100.0%).

219

**Production of pseudoviruses for the SARS-CoV-2 neutralization assay**

221   VSVΔG-luciferase-based viruses, in which the glycoprotein (G) gene has been replaced with

222   luciferase, were produced by transient transfection of viral glycoprotein expression plasmids

223   (pCG SARS-CoV-2 Spike, provided courtesy of Stefan Pölhmann[16], as well as pCAGGS VSV-G

224   or pCAGGS EboGP as controls) or no glycoprotein controls into HEK293T cells by TransIT-

225   2020. Briefly, cells were seeded into 15-cm culture dishes and allowed to attach for 24 hours

226   before transfection with 30 μg expression plasmid per plate. The transfection medium was

227   changed at approximately 16 hours post-transfection. The expression-enhancing reagent valproic

228   acid (VPA) was added to a final concentration of 3.75 mM, and the cells were incubated for

229   three to four hours. The medium was changed again, and the cells were inoculated with VSVΔG-

230   luc virus at a multiplicity of infection (MOI) of 0.3 for four hours before the medium was

231   changed again. At about 24 hours post-infection, the supernatants were collected and cleared of

232   debris by filtration through a 0.45-μm syringe filter.

233

**Antibody neutralization**

235   HEK293T cells were transfected with human ACE2 and TMPRSS2 by TransIT-2020. After 24

236   hours cells were plated into black 96-well tissue culture treated plates. Serum or plasma was

237   diluted to 1:20 followed by four subsequent 1:4 dilutions. Per well, 50 μl of pseudovirus

238   harboring either SARS-CoV-2 S, VSV-G or EboGP (adjusted to result in ~10,000 RLU in target

239   cells) was mixed with 50 μl of the respective serum or plasma dilution to give a final series of

240   longitudinal serum or plasma dilutions starting at 1:40 and incubated for one hour at 37°C.

241   Controls included wells with VSVΔG (no envelope), without added serum/plasma, and with

242   serum predetermined to possess or lack neutralizing activity. Subsequently, the 100 μl mix was

243   added to the target cells (performed in duplicate) and cells were incubated for 24 hours at 37°C.

244   Supernatants were then removed, cells were lysed, and luciferase activity was read as per

245   manufacturer instructions. Results were calculated as a percentage of no serum control. Each

246   plate was qualified by lack of infection with the no envelope control, and performance of

8

247     positive and negative controls. Non-linear regression curves and 80% neutralization titers

248     (NT80) were calculated in GraphPad Prism.

249

250     **Statistical analysis**

251     We calculated positive percent agreement (PPA), negative percent agreement (NPA), and overall

252     percent agreement (OPA) between the neutralizing antibody result and IgG, assuming IgG to be

253     the gold standard. We then calculated PPA, NPA, and OPA between the neutralizing antibody

254     result and IgM, assuming IgM to be the gold standard. We calculated 95% exact binomial

255     (Clopper-Pearson) confidence intervals for each proportion. IgG, IgM and NT80 levels were

256     non-normally distributed and were summarized using medians and interquartile ranges. We

257     compared antibody levels to dichotomously-defined clinical characteristics at various time points

258     using Wilcoxon rank sum tests. The correlations between age and IgG, IgM, and NT levels were

259     calculated using Spearman non-parametric correlation coefficients. Statistical calculations were

260     performed using python libraries scipy.stats, sklearn.metrics.auc and statsmodels.stats as well as

261     Stata v15.1 (College Station, TX).

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263

264

| Percentage of positive specimens from patients with positive SARS-CoV2 RT-PCR grouped by days since symptom onset and immune status | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **All Patient Samples** | | | | **Immunocompetent Only** | | | | **Immunocompromised only** | | | |
| **Assay** | **Total N** | **positive** | **%** | **95% CI** | **Total N** | **positive** | **%** | **95% CI** | **Total N** | **positive** | **%** | **95% CI** |
| **Architect SARS-CoV-2 IgG** | | | | | | | | | | | | |
| Day 1-7 | 41 | 12 | **29.3** | 23.7 - 35.6 | 35 | 10 | **28.6** | 22.5 - 35.5 | 6 | 2 | **33.3** | 16.1 - 55.3 |
| Day 8-14 | 106 | 68 | **64.2** | 60.5 - 67.7 | 82 | 53 | **64.6** | 60.4 - 68.7 | 24 | 15 | **62.5** | 53.5 - 70.7 |
| Day 15-21 | 113 | 102 | **90.3** | 87.7 - 92.3 | 77 | 72 | **93.5** | 90.5 - 95.6 | 36 | 30 | **83.3** | 77.1 - 88.1 |
| Day 22+ | 163 | 158 | **96.9** | 95.5 - 97.9 | 102 | 101 | **99** | 97.4 - 99.7 | 61 | 57 | **93.4** | 89.9 - 95.8 |
| All | 423 | 340 | **80.4** | 78.9 - 81.7 | 296 | 236 | **79.7** | 77.9 - 81.4 | 127 | 104 | **81.9** | 79.0 - 84.4 |
| **Architect SARS-CoV-2 IgM** | | | | | | | | | | | | |
| Day 1-7 | 26 | 10 | **38.5** | 30.6 - 47.0 | 22 | 9 | **40.9** | 32.1 - 50.4 | 4 | 1 | **25.0** | 6.9 - 54.4 |
| Day 8-14 | 91 | 68 | **74.7** | 70.9 - 78.1 | 70 | 54 | **77.1** | 72.8 - 80.9 | 21 | 14 | **66.7** | 56.9 - 75.2 |
| Day 15-21 | 83 | 75 | **90.4** | 87.2 - 92.8 | 53 | 49 | **92.5** | 88.4 - 95.2 | 30 | 26 | **86.7** | 79.9 - 91.5 |
| Day 22+ | 146 | 143 | **97.9** | 96.5 - 98.8 | 91 | 90 | **98.9** | 97.1 - 99.7 | 55 | 53 | **96.4** | 93.0 - 98.3 |
| All | 346 | 296 | **85.5** | 84.1 - 86.9 | 236 | 202 | **85.6** | 83.7 - 87.2 | 110 | 94 | **85.5** | 82.5 - 87.9 |
| **Antibody Neutralization Assay** | | | | | | | | | | | | |
| Day 1-7 | 10 | 4 | **40.0** | 26.1 - 55.5 | 9 | 3 | **33.3** | 19.6 - 50.2 | 1 | 1 | **100** | 25.0 - 100 |
| Day 8-14 | 24 | 14 | **58.3** | 49.4 - 66.8 | 18 | 12 | **66.7** | 55.9 - 76.0 | 6 | 2 | **33.3** | 16.1 - 55.3 |
| Day 15-21 | 10 | 7 | **70.0** | 54.2 - 82.4 | 6 | 5 | **83.3** | 61.1 - 95.3 | 4 | 2 | **50.0** | 24.3 - 75.7 |
| Day 22+ | 14 | 13 | **92.9** | 81.9 - 98.0 | 9 | 9 | **100** | 85.7 - 100 | 5 | 4 | **80.0** | 54.6 - 94.4 |
| All | 58 | 38 | **65.5** | 60.3 - 70.4 | 42 | 29 | **69.0** | 62.8 - 74.7 | 16 | 9 | **56.2** | 44.8 - 67.1 |

266

**Table 1: Clinical sensitivities of the Abbott Architect SARS-CoV-2 IgG and IgM and *in vitro* neutralization assays**

Clinical sensitivity of each assay, defined as the percent of samples from RT-PCR confirmed SARS-CoV-2 infected patients that test positive in each assay. Total numbers of samples, positive samples, and percent positive among total samples with 95% confidence intervals (CI) are shown for the indicated time frames for samples from all patients (left column), samples from immunocompetent patients only (middle column), and samples from immunocompromised patients only (right column.) Immunocompromised patients: six solid organ transplant recipients on tacrolimus and MMF and one rheumatoid arthritis patient on methotrexate and infliximab.

273    **FIGURE LEGENDS**

274

275    **Figure 1: Seroprevalence of Antibodies to SARS-CoV-2**

276    **(A)** Schematic of testing performed and location of patient populations assessed. **(B)** IgG S/C

277    ratios for SARS-CoV-2 PCR-positive patient samples for the indicated weekly timeframes post-

278    onset of symptoms (if multiple samples per patient were collected, the sample with the highest

279    S/C value within each time frame is plotted). The percent of patients with positive antibody

280    responses measured within each timeframe is indicated below the graphs. **(C)** IgG S/C ratios

281    measured in pre-COVID samples; specificity and number of samples is indicated on graph. **(D)**

282    Receiver operating characteristic (ROC) curves for IgG levels for all samples from SARS-CoV-2

283    PCR-positive patients within the indicated weekly time frames. AUCs for are 0.537 (day 1-7),

284    0.827 (day 8-14), 0.946 (day 15-21), 0.990 (day 22+). **(E)** IgM S/C ratios, as in **(B)**. **(F)** IgM S/C

285    ratios measured in pre-COVID samples. (**G**) ROC curves for IgM levels, as in (**D**); AUCs are

286    0.720 (day 0-7), 0.955 (day 8-14), 0.970 (day 15-21), 0.999 (day 22+). IgG **(H)** and IgM **(I)** S/C

287    ratios were determined for hospitalized patients and outpatients and blood donors on whom

288    SARS-CoV-2 PCR testing was positive or negative or was not performed. Numbers of

289    seroreactive and total individuals tested are shown in tables below the graphs. The circled data

290    points in (**H**) were additionally tested by the VITROS and neutralization assays. For patients

291    with multiple samples, the single highest S/C value is plotted. In (**B**), (**C**), and (**H**), the dotted

292    line at 1.4 indicates cutoff for IgG positivity; in (**E**), (**F**), and (**I**), the dotted line at 0.6 indicates

293    cutoff for IgM positivity; data points in black and gray are above and below the indicated

294    cutoffs, respectively.

295

296    **Figure 2: Longitudinal dynamics and *in vitro* neutralizing activity of antibodies against**

297    **SARS-CoV-2**

298    (A) IgG and IgM levels for SARS-CoV-2 PCR positive matched patient samples. Percent of data

299    points in each quadrant and positive percent agreement (PPA), negative percent agreement

300    (NPA), and overall percent agreement (OPA) between IgG and IgM are shown. 80%

301    neutralization titers (NT80) plotted against IgM (**B**) and IgG (**C**) S/C values. The cutoff for

302    NT80 was a titer level of >40; negative results are non-numeric (<40) and are plotted at 35 for

303    visualization purposes.  (**D-F**), IgM (**D**) and IgG (**E**) S/C ratios and NT80 titers (**F**) for SARS-

1

304 CoV-2 PCR-positive patients were plotted against day post symptom onset.

305 Immunocompromised patients are shown in blue. In (**D, E**), for patients with multiple same-day

306 samples, the sample with the highest S/C value is plotted. **(G)** For the 6 SARS-CoV-2 PCR-

307 positive patients whose IgM, IgG, and NT80 seroconversion events were captured during serial

308 sampling, the days post-symptom onset seroconversion events are compared. **(H)** NT80 activity

309 was evaluated per patient for the indicated time frames post onset of symptoms. The percent of

310 patients with detectable NT80 activity measured within each time frame is indicated below the

311 graphs. If multiple samples per patient were collected, the sample with the highest NT80 value

312 within each time frame was used. (**I**) The average NT80 activity (right axis) and IgG and IgM

313 (left axis) levels are plotted by day post-symptom onset (left); corresponding graphs for

314 individual patients are shown in a 3x3 grid (right). If multiple samples per patient were collected,

315 the sample with the highest S/C or NT80 value per time frame was used.

316

317 **Table S1:** Baseline demographic characteristics, presenting symptoms, chronic medical

318 conditions, medications, and radiographic findings of 43 SARS-CoV-2 PCR-positive UCSF

319 outpatients and hospitalized patients.

320

321 **Table S2:** Descriptive demographic characteristics of individuals who donated blood at San

322 Francisco Bay Area community blood centers (Vitalant Research Institute).

323

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**Competing Interests**

370 CYC is the director of the UCSF-Abbott Viral Diagnostics and Discovery Center (VDDC) and

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372 employees of Abbott Laboratories. The other authors have no competing interests to declare.

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**Contributions**

375 C.Y.C. conceived, designed, and supervised the study. D.L.N., and G.M.G. coordinated the

376 study. D.L.N., G.M.G., B.R.S., A.G.L., S.P.B., J.B., and C.Y.C. analyzed data, designed figures,

377 and wrote and edited the manuscript. A.S.G., V.S., C.S.S.M., A.G., D.R.G., E.H., W.G., Y.A.S.,

378 C.W., K.R., J.H., F.A., L.P., C.-Y.O., C.M.L. contributed to the collection of clinical specimens.

379 K.S., T.K., and E.C.T. coordinated clinical sample collection and IgG testing. S.M. provided

380 clinical data and facilitated sample collection. L.M.H., K.T., N.A., D.N.N., N.M.N., and D.Q.

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381    performed chart review. M.S., B.C., V.G., P.W., M.B., and J.D.W. coordinated blood donor

382    samples and data. S.Z., L.D., G.S., and S.K.P. performed neutralizing antibody assays. J.M.C.H.,

383    J.P., M.R., K.C., and S.P. performed IgG and IgM testing and provided data establishing testing

384    characteristics of SARS-CoV-2 IgG and IgM assays. N.K.H. performed biostatistical analysis

385    and review. All authors read the manuscript and agreed to its contents.

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396    **Data Availability**

397    Raw data used in this study, including de-identified patient metadata and test results, are

398    available upon request.

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