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1 **Detection of Nucleocapsid Antibody to SARS-CoV-2 is More Sensitive than Antibody to**

2 **Spike Protein in COVID-19 Patients**

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24    **Footnotes**

28

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30

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39    **ABSTRACT**

40    **Background:** SARS-CoV-2, the cause of coronavirus disease 2019 (COVID-19), is associated

41    with respiratory-related morbidity and mortality.  Assays to detect virus-specific antibodies are

42    important to understand the prevalence of infection and the course of the immune response.

43    **Methodology:**  Quantitative measurements of plasma or serum antibodies by luciferase

44    immunoprecipitation assay systems (LIPS) to the nucleocapsid and spike proteins were analyzed

45    in 100 cross-sectional or longitudinal samples from SARS-CoV-2-infected patients.  A subset of

46    samples was tested with and without heat inactivation.

47    **Results:** Fifteen or more days after symptom onset, antibodies against SARS-CoV-2

48    nucleocapsid protein showed 100% sensitivity and 100% specificity, while antibodies to spike

49    protein were detected with 91% sensitivity and 100% specificity.  Neither antibody levels nor the

50    rate of seropositivity were significantly reduced by heat inactivation of samples.  Analysis of

51    daily samples from six patients with COVID-19 showed anti-nucleocapsid and spike antibodies

52    appearing between day 8 to day 14 after initial symptoms. Immunocompromised patients

53    generally had a delayed antibody response to SARS-CoV-2 compared to immunocompetent

54    patients.

55    **Conclusions:**  Antibody to the nucleocapsid protein of SARS-CoV-2 is more sensitive than

56    spike protein antibody for detecting early infection.  Analyzing heat-inactivated samples by LIPS

57    is a safe and sensitive method for detecting SARS-CoV-2 antibodies.

58

59    Keywords: COVID-19; SARS-CoV-2; coronavirus; serology

60

61

62    Infections with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing

63    coronavirus disease 2019 (COVID-19), were first reported in China [1-4].  The major clinical

64    feature of COVID-19 SARS-CoV-2 is virus-associated pneumonitis [5-7]. Unlike other highly

65    pathogenic coronaviruses such as SARS/SARS-CoV-1 and Middle East respiratory syndrome

66    coronavirus (MERS-CoV) [8], SARS-CoV-2 spreads more rapidly and reached six of the seven

67    continents, including North America [9], within three months of the initial outbreak.  Nucleic

68    acid-based testing of oropharyngeal or nasopharyngeal swabs and saliva is useful for diagnosing

69    acute infection.  SARS-CoV-2 virus RNA can often be detected in upper respiratory secretions at

70    the time of the first appearance of symptoms, peaks during the first week, and later declines with

71    time [10, 11].  RNA from SARS-CoV-2, like the related SARS-CoV-1 [12], can also be detected

72    in blood [11, 13], and high levels of circulating viral RNA are associated with more severe

73    disease [13].

74        Assessment of the antibody response to SARS-CoV-2 should complement the RNA-

75    based tests and improve our understanding of the disease course, contribute to epidemiological

76    studies and inform vaccine development.  Antibodies to the nucleocapsid protein are the most

77    sensitive target for serologic diagnosis of infection with SARS-CoV-1 [14, 15].  Antibodies

78    against the spike protein of SARS-CoV-1, the target of neutralizing antibody and vaccine

79    development, emerge at a later time than those against the nucleocapsid protein.  Recently,

80    several groups have reported serological diagnostic tests using the nucleocapsid and/or spike

81    protein from SARS-CoV-2 by ELISA [11, 16, 17], immunofluorescence [18] and even a lateral

82    flow test [19].  One study that used ELISA to measure only antibodies to the nucleocapsid

83    protein found that patients become seropositive 10-18 days after the onset of symptoms [16].  A

84    commercial ELISA using the spike protein demonstrated that IgG antibodies were detectable at a

85    median of 14 days after onset of symptoms [17].  To et al. examined antibodies against both the

86    spike and nucleocapsid by ELISA in a small number of samples and found that IgG antibodies

87    against the nucleocapsid protein were generally detectable at about the same time as antibodies

88    to the spike protein [11].  Despite these findings, further studies are needed to better understand

89    antibody dynamics in persons infected with SARS-CoV-2 to determine the most sensitive and

90    specific antibody assays, and to use these antibody-based tests to determine seroprevalence in

91    different populations. In addition, it is currently unknown whether the viral RNA that has been

92    detected in the blood [11, 13] indicates the presence of infectious virus, but this has the potential

93    to be a safety hazard for health care workers, clinical laboratory technicians and researchers

94    analyzing serology in persons infected with SARS-CoV-2. Thus, a sensitive and specific

95    antibody assay using heat treated plasma or serum may enhance safety when working with these

96    fluids.

97    We and others have employed a liquid phase immunoassay technology termed Luciferase

98    Immunoprecipitation Systems (LIPS) to measure antibodies against many different viruses, to

99    stratify infected patients based on the level of their antibodies, and for virus discovery [20].

100    LIPS has shown promise for detecting antibodies against coronaviruses including the

101    nucleocapsid of MERS-CoV [21] and the spike protein of swine acute diarrhea syndrome

102    coronavirus (SADS-CoV) [22]. Unlike ELISA, which is solid phase, LIPS is performed in

103    solution, thus maintaining the native antigen conformation.  The antigen is produced in

104    mammalian cells and often retains post-translational modifications of the antigen, unlike

105    bacterial recombinant proteins or peptide based ELISAs.  LIPS assays typically have a dynamic

106    range up to 6 $\log_{10}$ for some antigens and require < 5 ul of plasma or sera for testing. Here

107    recombinant nucleocapsid and spike protein from SARS-CoV-2 as antigens in LIPS assays were

5

108    used to measure antibodies in patients with COVID-19 from four geographically disparate

109    locations across the United States. The LIPS assay showed high sensitivity and specificity for

110    detecting SARS-CoV-2 antibodies and demonstrated that nucleocapsid antibodies emerge before

111    spike antibodies.  Moreover, as there are potential safety issues related to the presence of SARS-

112    CoV-2 RNA in blood, we show that heat inactivation of plasma at 56˚C for 30 min does not

113    significantly reduce the sensitivity of the LIPS assay and thus allows testing to be performed

114    more safely.

115    **METHODS**

116    **Characteristics of the patients with COVID-19**

117    This retrospective study analyzed both cross-sectional and longitudinal blood samples collected

118    from patients with COVID-19 or controls from four clinical sites. Anonymized plasma or serum

119    from patients from University of California, San Diego (UCSD, n=3), University of Washington,

120    Seattle (UW, n=17), EvergreenHealth, Kirkland, Washington (EH, n=23) (**Table 1**) were

121    obtained under an IRB exemption. Plasma from patients at the NIH Clinical Center, NIH (n=6)

122    were obtained under a protocol approved by the IRB of the NIH Intramural Research Program;

123    all patients signed consent.  Additional anonymized blood bank donor controls (n=32) collected

124    at the NIH Clinical Center prior to 2018 were used as uninfected controls for serological testing.

125    The time interval between the initial symptoms and obtaining plasma/serum samples from PCR+

126    confirmed cases was variable and ranged from 2 to 50 days. SARS-CoV2 infection was

127    confirmed in each case by reverse transcriptase PCR detection of viral RNA from nasal and/or

128    throat swabs performed at clinical laboratories associated with each location.  Thirteen patients

129    from the UW, 13 of 23 subjects from the outbreak at EH (including the nursing home and family

130    members of health workers), 3 patients from the UCSD (two samples from each), and 6 patients

6

131    from the NIH Clinical Center, Bethesda, MD were confirmed positive for SARS-CoV-2 RNA. In

132    the case of the NIH samples, serial daily blood drawn samples (n=68) were available covering 0-

133    20 days from symptom onset.

134    **Storage and Heat Inactivation**

135    Plasma/serum samples were collected and stored frozen at -80º C, except for the heat-inactivated

136    samples from the NIH that were not previously frozen.  In light of previous studies that showed a

137    marked loss in infectivity of  SARS-CoV-1 [23] and MERS [24] coronaviruses with heating, we

138    adopted a precautionary safety protocol performed before analysis.  An aliquot of plasma/serum

139    from each patient sample was first incubated at 56º C for 30 min and then used for testing as

140    described below.

141

142    **Luciferase Immunoprecipitation Systems (LIPS) for Measurement of SARS-CoV-2**

143    **Antibodies**

144    LIPS assays, in which viral proteins fused to light-emitting luciferase  are immunoprecipitated,

145    were essentially performed as described [25].  A plasmid expressing the nucleocapsid of SARS-

146    CoV-2 (amino acids 1-417 of GenBank MN908947) was generated as a synthetic DNA (Twist

147    Biosciences) and cloned into the pREN2 eukaryotic expression vector as C-terminal *Renilla*

148    luciferase fusion protein. A plasmid expressing the spike protein of SARS-CoV-2 (amino acids

149    1-538 of GenBank MN908947) was generated by PCR from a plasmid containing a prefusion

150    form of the spike protein (2019-nCoV-2_S-2P [26]) and produced as a N-terminal fusion protein

151    in the pGAUS3 vector for expression as a *Gaussia* luciferase fusion protein.  The resulting

152    plasmid was termed pGAUS3-Spike.  A second spike construct, pGAUS3-Spike-Δ2 (amino

153    acids 1-513) was also constructed in the pGAUS3 vector in the same way.  Preliminary tests

154    comparing antibody detection using pGAUS3-Spike-Δ2 and pGAUS3-Spike showed similar

155    results and the former construct was not used further.

156         Nucleocapsid and spike protein-light emitting plasmid constructs were transfected into

157    Cos1 cells with Fugene-6 and lysates were harvested 48 hours later to obtain crude cell extracts.

158    For testing, heat-inactivated serum or plasma samples were diluted 1:10 in assay buffer A

159    (20 mM Tris, pH 7.5, 150 mM NaCl, 5 mM MgCl$_2$, 1% Triton X-100) and 10 μl of the diluted

160    sample were then tested in a 96-well microtiter plate as described  [25].  After incubation at room

161    temperature for one hour, the mixture was transferred to a microtiter filter plate containing

162    protein A/G beads and incubated for one hour. The antibody-antigen-bead complexes were then

163    washed eight times with buffer A and twice with PBS on a microtiter filter plate to remove

164    unbound antigens. After the final wash coelenterazine substrate (Promega) was added to detect

165    *Renilla* luciferase and *Gaussia* reporter activity and light units (LU) were measured in a Berthold

166    LB 960 Centro microplate luminometer (Berthold Technologies, Bad Wildbad).

167         Antibody levels were reported as the geometric mean level (GML) with 95% confidence

168    interval (CI). Cut-off limits for determining positive antibodies in the SARS-CoV-2-infected

169    samples were based on the mean plus three standard deviations of the serum values derived from

170    the 32 uninfected blood donor controls or by receiver operator characteristics (ROC) analysis.

171    For some of the data percentages for categorical variables, mean and range, geometric mean plus

172    95% CI were used to describe the data. Wilcoxon signed rank were used for statistical analysis.

173

174    **RESULTS**

175    **Characteristics of the patients with COVID-19**

8

176    Patients with COVID-19 were located in four geographically distinct locations across the United

177    States and included 35 SARS-CoV-2 cases confirmed by PCR, 10 subjects with COVID-19-like

178    symptoms or household contacts of persons with COVID-19 (not tested by PCR), and 32 blood

179    donors who donated samples before 2018 used as controls (Table 1). The majority of the SARS-

180    CoV-2 PCR-confirmed cases were male (87%) and the median age was 44 years (range, 32-50

181    years).  A subset of the SARS-CoV-2 PCR-confirmed cases had one or more risk factors

182    including heart disease, lung disease, diabetes, and/or they were immunocompromised.  Two

183    different plasma samples, drawn 2-3 days apart, were available for each of the three COVID-19

184    cases from the UCSD and multiple daily samples were available from the NIH patients with

185    COVID-19.  Combining the cross-sectional and longitudinal studies resulted in 100 samples

186    from PCR+ patients.

187

188    **Detection of Antibodies to the Nucleocapsid Protein of SARS-CoV-2 is More Sensitive than**

189    **Antibodies to the Spike Protein in COVID-19 Patients**

190    LIPS assays for detecting antibodies were developed using SARS-CoV-2 nucleocapsid and spike

191    antigens produced in mammalian cells. Pilot experiments using nucleocapsid-*Renilla* luciferase

192    and spike protein-*Gaussia* luciferase fusion proteins were conducted with serum or plasma from

193    blood donor controls collected prior to 2018. Results showed a low background with little or no

194    antibody immunoreactivity against the spike protein, but there was a higher background

195    immunoreactivity against the nucleocapsid (**data not shown**). Based on the need to develop a

196    highly specific SARS-CoV-2 LIPS serological test without potential false positives, stringent

197    cut-off values from the blood donor controls were assigned based on statistical methods and/or

198    ROC. From this analysis, cut-off values for the nucleocapsid and spike proteins were derived

199    from the mean plus four standard deviations (125,000 LU) and the mean plus three standard

200    deviations (45,000 LU) of the blood donor controls, respectively.

201       Using these cut-off values, plasma or serum samples from the COVID-19 cohort were

202    evaluated by LIPS assay for antibodies against the nucleocapsid or spike protein. For safety

203    reasons, all samples used for this analysis were heated at 56°C for 30 min to reduce the

204    likelihood of having infectious virus in the samples. Coded plasma or serum samples from

205    suspected COVID-19 cases from EH were then tested as well as noncoded pre-2018 blood

206    donors, and SARS-CoV-2 PCR-positive cases from the UCSD, UW, EH, and the NIH Clinical

207    Center (NIH). A wide dynamic range of antibody levels against the nucleocapsid and spike

208    protein were observed differing by up to 100-fold between samples (**Figure 1**). In order to

209    compare the sensitivity of the nucleocapsid and spike LIPS assays, a minimum interval of >14

210    days between onset of symptoms and time of blood collection was used to determine the number

211    of seropositive serum or plasma samples in the SARS-CoV-2 PCR-positive group. Among the

212    PCR+ patient samples collected >14 days after onset of symptoms (**Figure 1, black dots**),

213    seropositive nucleocapsid antibodies were detected in 4/4 patients from UW, 7/7 from EH, and

214    32/32 serial samples from the six NIH patients, yielding both a sensitivity and specificity of

215    100%. A similar analysis of the spike antibody in samples collected >14 days after onset of

216    symptoms showed a slightly lower sensitivity of 91% (32/35) with 100% specificity, where 4/4

217    patients from UW, 6/7 from EH, and 22/24 from NIH were seropositive.

218       Evaluation of samples collected at ≤14 days after onset of symptoms showed reduced

219    sensitivity, but specificity was maintained. The sensitivity for antibody to the nucleocapsid

220    protein at this time point was 51% (33/65) with antibodies detected in 1/6 samples from UCSD,

221    5/9 from UW, 3/6 from EH and 24/44 from NIH (**Figure 1, orange dots**). Analysis of spike

222     antibodies of samples collected at ≤14 days after onset of symptoms showed a sensitivity of 43%

223     (28/65) with antibody detected in 0/6 samples from UCSD, 4/9 samples from UW, 2/6 from EH

224     and 22/44 from NIH.   Taken together, our findings indicate that detection of antibodies against

225     the nucleocapsid protein is more sensitive than detection of antibodies against the spike protein,

226     and that nucleocapsid antibodies generally appear earlier than spike antibodies.

227     In addition to the SARS-CoV-2 PCR-confirmed patients, suspected cases of COVID-19

228     from EH were also analyzed for seropositivity. Nine of the ten suspected cases without viral PCR

229     confirmation, that showed symptoms compatible with COVID-19 collected between January and

230     February 2020, were seronegative for both nucleocapsid and spike antibodies (**Figure 1**).

231     Interestingly, one case from March 2020 from a person who was a household contact with a

232     SARS-CoV-2 PCR+ patient, was seropositive for both nucleocapsid and spike antibodies.

233     Since there is interest in using serological assays to assess current and historical

234     infections, we evaluated the robustness of the LIPS assay for detecting SARS-CoV-2 antibodies

235     by analyzing the level of antibodies in all PCR-confirmed samples collected more than 14 days

236     after symptom onset. The geometric mean level (GML) of nucleocapsid antibody levels in the 35

237     seropositive samples was 694,600 (95% CI, 570,000-844,600 LU), which was approximately 32

238     times higher than the GML of the blood donor controls of 21,356 LU (95% CI, 17,032-

239     26,752).   Antibodies against spike protein showed a similar discriminatory potential for the

240     seropositive samples with GML of 346,800 LU (95% CI, 218,800-550,000 LU), which was

241     approximately 21 times higher than the blood donor controls with 16,843 LU (95% CI, 14,172-

242     20,007 LU).   These findings indicate that the LIPS assays for antibodies to nucleocapsid and

243     spike protein are robust and should be useful to evaluate the prevalence of infection with.

244     SARS-CoV-2.

11

245

246 **Time course of the appearance of serum antibodies against SARS-CoV-2 differs in**

247 **immunocompetent and immunocompromised patients**

248 To understand the timing and trajectory of SARS-CoV-2 antibodies against nucleocapsid and

249 spike proteins, serial daily blood samples from the six NIH patients with COVID-19 were

250 studied.  In all six subjects, SARS-CoV-2 antibody levels rose with time in both the three

251 immunocompetent (**Figure 2A**, NIH patients 1-3) and three immunocompromised patients

252 (**Figure 2B**, NIH patients 4-6).  These latter three patients had chronic lymphocytic leukemia,

253 metastatic chordoma, or had received a hematopoietic stem-cell transplant.   All three

254 immunocompetent COVID-19 patients showed a rapid rise in antibody to nucleocapsid and

255 began within 10 days of symptom onset in 2 patients (no samples were available before day 11

256 for the third patient, **Figure 2A)**.  Antibodies against the spike protein in these three

257 immunocompetent patients generally tracked with the nucleocapsid antibodies, but in one case

258 seropositivity appeared 2 days later than nucleocapsid antibody.  The third patient, NIH-3, with a

259 history of hypertension and heart disease died of cardiovascular shock and hypoxemia 13 days

260 after onset of symptoms.

261 Antibody profiles in the three immunocompromised NIH patients showed more blunted

262 responses against the SARS-CoV-2 antigens (**Figure 2B**).  Patient NIH-4 became seropositive

263 for both nucleocapsid and spike antibodies on day 14 and these antibodies then plateaued at these

264 low levels for the next seven days. Similarly, patient NIH-5 did not become seropositive until

265 day 13 for spike antibody and day 14 for nucleocapsid antibody.  Patient NIH-6 was both PCR+

266 for SARS-CoV-2 and seropositive on the day of symptoms, suggesting that he had an

267 asymptomatic infection for several days before diagnosis.  Despite the blunted antibody

268    response, none of the immunocompromised patients died. Overall, the results with this small

269    group of patients suggests that immunocompromised patients generally have a more attenuated

270    and/or delayed antibody response to SARS-CoV-2 than immunocompetent patients.

271

272    **Heat inactivation of plasma minimally impacts detection of antibody to SARS-CoV-2 in the**

273    **LIPS assay**

274    While heating plasma to $56^{\circ}$ C for 30 min has been shown to reduce the titer of human

275    coronaviruses, heating  might reduce or eliminate IgM and IgG responses [27].  Therefore, we

276    performed LIPS assays on a subset of the patients with COVID-19 from the known or suspected

277    cases (N=38) with and without heat inactivation, to evaluate its impact on nucleocapsid antibody

278    levels and seropositivity status.  Evaluation of antibody responses in heated versus unheated

279    plasma samples showed that antibody levels were mostly unchanged (**Figure 3**).  In a single

280    sample from one patient with COVID-19, antibody to SARS-CoV-2 was not detected after heat-

281    inactivation. Of note, this sample came from an NIH patient with COVID-19 who was antibody

282    positive at day 7 using non-heated plasma and became seropositive using heat-inactivated plasma

283    from day 8.  Statistical analysis showed no significant difference in antibody levels between

284    plasma that was heated or unheated (Wilcoxon Signed rank test) and the values were highly

285    correlative (Rs=0.913; P<0.0001).  These findings indicate that the heat-inactivation process is

286    diagnostically suitable for testing of SARS-CoV-2 antibodies by LIPS.

287

288    **DISCUSSION**

289          We used a fluid-phase LIPS assay to investigate antibodies to the SARS-CoV-2

290    nucleocapsid and spike protein in COVID-19 patients after infection.  The LIPS assay

291    demonstrated high sensitivity and a wider dynamic range for antibody detection compared to

13

292  other published assays [11, 16-19].  An analysis of longitudinal plasma samples showed that

293  antibodies against the nucleocapsid and spike proteins appeared about the same time between

294  day 8 and day14 after the onset of symptoms.  Only one study to date has examined antibodies

295  separately against the nucleocapsid protein and spike protein [11] and our findings are in general

296  agreement. COVID-19 patient plasma samples obtained ≥14 days after symptom onset showed

297  that the LIPS assay for antibodies against the nucleocapsid and spike protein had 100% and 94%

298  sensitivity, respectively, with 100% specificity for both antibodies.  Additional studies using this

299  high-throughput, highly quantitative LIPS assay may also help determine whether the relative

300  levels of antibodies observed in convalescent COVID-19 patients or uninfected vaccinated

301  persons correlate with prevention of reinfection or primary infection, respectively. Quantitative

302  antibody profiles will be useful in determining antibody decay over time.  It is known that for

303  some viral infections there is long-lasting antibody responses and protection from infection, but

304  for others antibody levels wane at faster rates [28]. Following humoral response profiles of

305  natural infection from convalescent COVID-19 cases over time should provide important

306  insights into the half-life of these antibodies.

307  Using the quantitative LIPS assay, our studies with serial patient samples from the NIH

308  cohort showed the temporal relationship between antibody dynamics with onset of symptoms

309  and PCR positivity for SARS-CoV-2.  Cut-off values for a positive result was based on pre-2018

310  blood donors and may underestimate the number of seropositive persons because some

311  individual patients showed low antibody values initially that gradually rose before exceeding the

312  cut-off value.  Nevertheless, all three of the immunocompetent COVID-19 patients showed rapid

313  seroconversion within 10 days of onset of symptoms for antibody to the nucleocapsid protein and

314  robust, but slightly delayed for antibody to the spike proteins. In contrast, the

14

315 immunocompromised NIH patients exhibited a slower rise in antibody levels with a plateau at

316 lower levels compared to the immunocompetent patients, and two patients did not become

317 seropositive until 14 days after onset of symptoms. Nonetheless, the immunocompromised

318 patients had a favorable clinical outcome. The NIH patient who died (NIH-3) was not

319 immunocompromised and had a rapid rise in antibody production reaching levels comparable to

320 the other immunocompetent patients. In addition, one of the two EH patients who died showed

321 the highest antibody levels in that cohort of patients. While excessive proinflammatory responses

322 to the virus have been reported to contribute to poor outcomes [29-31], larger studies of COVID-

323 19 patients are required to determine whether antibody levels directly correlate with disease

324 severity.

325 Prior studies have shown high levels of SARS-CoV-2 RNA in blood from patients with

326 COVID-19 [1, 4]. At present, it is not certain whether infectious virus might be circulating in the

327 blood early during infection. Accordingly, we heated plasma or serum to 56º C for 30 min to

328 reduce the titer of SARS-CoV-2 before performing the LIPS assays, since prior studies have

329 shown a marked loss in infectivity of SARS-CoV-1 [23, 32] and MERS [24] coronaviruses with

330 heat treatment. While impaired detection of viral IgM and IgG antibody responses to viruses

331 after heating samples to 56ºC has been reported [27], and several abstracts report similar findings

332 with SARS-CoV-2 samples, our direct comparison of untreated and treated samples found high

333 concordance of the antibody values revealing the suitability of heat inactivation. This

334 inactivation protocol may be useful to enhance safety when studying highly infectious saliva

335 from COVID-19 patients [11] for IgG and IgA antibodies. Further modification of LIPS assays

336 for detection of SARS-CoV-2 antibodies, including the use of different protein fragments, full-

337 length spike protein, and/or different luciferase reporters, may further improve assay

338    performance. Nonetheless, our current assay provides highly quantitative results with a high

339    degree of sensitivity and specificity and should be useful for larger seroepidemiologic studies.

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345

346

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418 **Figure legends**

419 **Figure 1. Detection of antibodies against SARS-CoV-2 nucleocapsid and spike protein in**

420 **patients with COVID-19.** Antibody levels against SARS-CoV-2 nucleocapsid and spike

421 protein were determined in 32 pre-2018 blood donors, 10 suspected COVID-19 cases (not PCR

422 confirmed) from EvergreenHealth, Kirkland, WA (EH), three PCR+ COVID-19 patients from

423 UCSD, 13 PCR+ COVID-19 patients from the University of Washington (UW), 13 PCR+

424 COVID-19 patients from EH, and 6 COVID-19 patients from the NIH Clinical Center (NIH).

425 Each symbol represents a sample from an individual patient or different time points from an

426 individual patient. Antibody levels are plotted in light units (LU) on a $\log_{10}$ scale. Black circles

427 represent plasma or serum samples obtained after 15 or more days after symptom onset and

428 orange circles are from plasma or serum samples obtained 14 or less days after symptom onset.

429 The dashed lines represent the cutoff level for determining positive antibody titers as described

430 in the Methods.

431

432 **Figure 2. Longitudinal profile of antibodies against nucleocapsid and spike protein in**

433 **immunocompetent and immunocompromised COVID-19 patients from NIH.** Antibody

434 levels were determined in daily blood draws from six COVID-19 patients. Three of the COVID-

435 19 patients were immunocompetent (**Panel A, NIH-1-3**) and three (**Panel B, NIH-4-6)** were

436 immunocompromised. The levels of antibody to the nucleocapsid (black line) and spike protein

437 (blue line) over time are shown and were plotted on the y-axis using a $\log_{10}$ scale. Time zero

438 represents the first day symptoms appeared, and the vertical arrows are the time of diagnosis by

439 PCR. The cut-off values for determining seropositivity is shown by the dotted lines. The red X's

440 indicates the day after onset of symptoms that patient NIH-3 died.

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442    **Figure 3.  Heat inactivation of plasma or serum samples has no significant impact on**

443    **detection of nucleocapsid antibodies.** A subset (n=38) of plasma samples from patients with

444    COVID-19 including samples from PCR-positive patients from very early infection (less than 8

445    days) and at later times after initial infection were analyzed.  Levels of antibody to the

446    nucleocapsid protein were determined by LIPS for aliquots of paired samples from unheated

447    plasma or serum and from heated plasma or serum. Antibody levels were plotted, and the

448    horizontal and vertical dotted lines represent the cutoff values for seropositivity. The diagonal

449    line is a theoretical value if the antibody levels were identical for heated and unheated samples.

450    The antibody values strongly correlated for heat treated and not heat-treated samples as shown

451    by the Spearman rank correlation ($Rs$) of 0.92 ($P$ <0.0001) and only one sample showed a

452    significant decrease with heating.

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**Table 1. Subject Characteristics of COVID-19 Cohort**

| | N | Gender (M:F) ratio | Age years (range) | With one or More Risk Factors* no. (%) | SARS-CoV-2 PCR Positive no. (%) | Time from Symptoms to First Blood Draw Average (range) | Ventilator no. (%) | Mortality no. (%) |
|---|---|---|---|---|---|---|---|---|
| Blood Donors | 32 | ND | ND | ND | ND | ND | ND | ND |
| Suspected Cases[#] | 10 | 4:6 | 32(7-49) | 0 (0)[‡] | 0 (0)[§] | 47.1 Days (26-79)[‡] | 0 (0) | 0 (0) |
| Univ. Calif., San Diego | 3 | 2:1 | 73 (59-84) | 2 (66) | 3 (100) | 7.8 Days (5-14) | 3 (100) | 1 (33) |
| Univ. of Washington | 13 | 10:3 | 66 (43-95) | 13 (100) | 13 (100) | 13.2 Days (4-24) | 4 (31) | 5 (38) |
| EvergreenHealth | 13 | 3:10 | 59 (19-88) | 6 (46) | 13 (100) | 18 Days (2-50)[‡] | 3 (23) | 3 (23) |
| NIH Clinical Center | 6 | 5:1 | 45 (22-67) | 5 (83) | 6 (100) | 5.5 Days (0-11) | 3 (50) | 1 (17) |

*Risk factors including heart disease, lung disease, diabetes, obesity, and/or immunocompromise

Abbreviation: ND, not determined

[#]EvergreenHealth

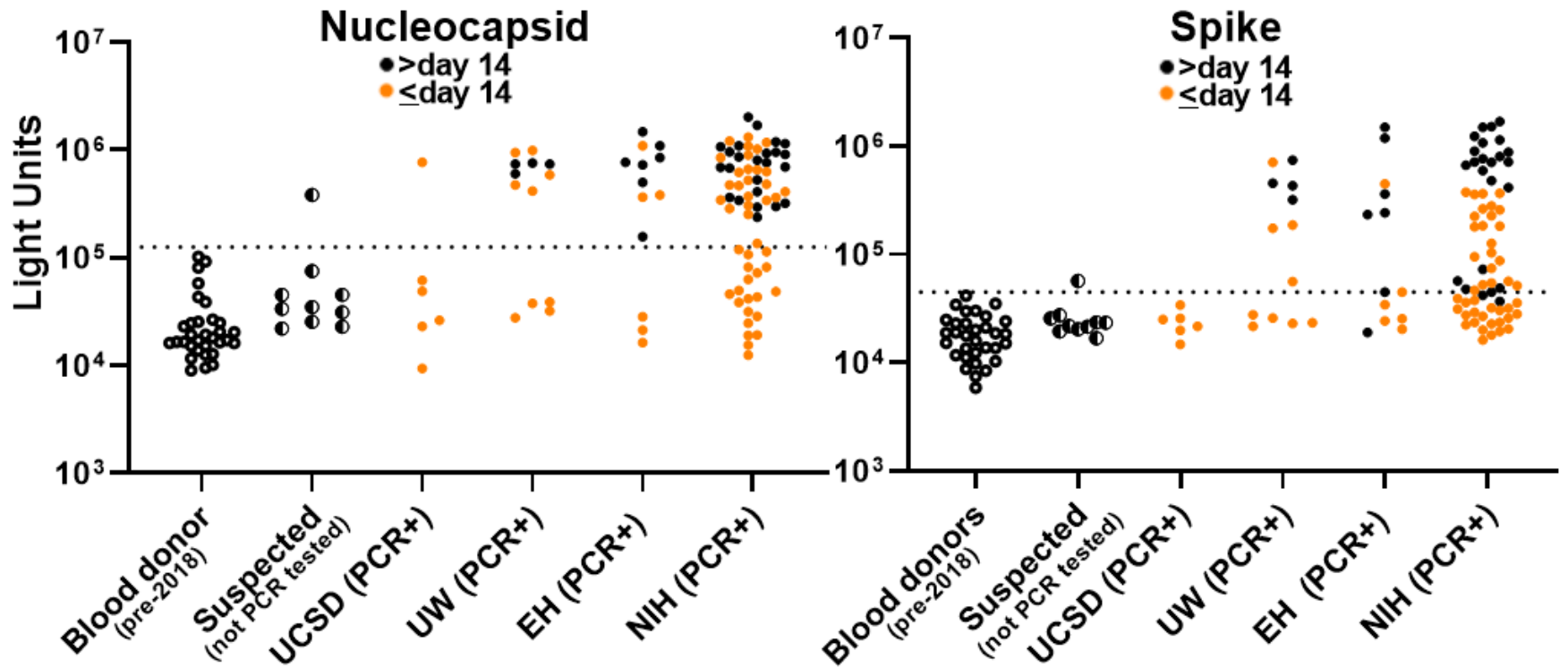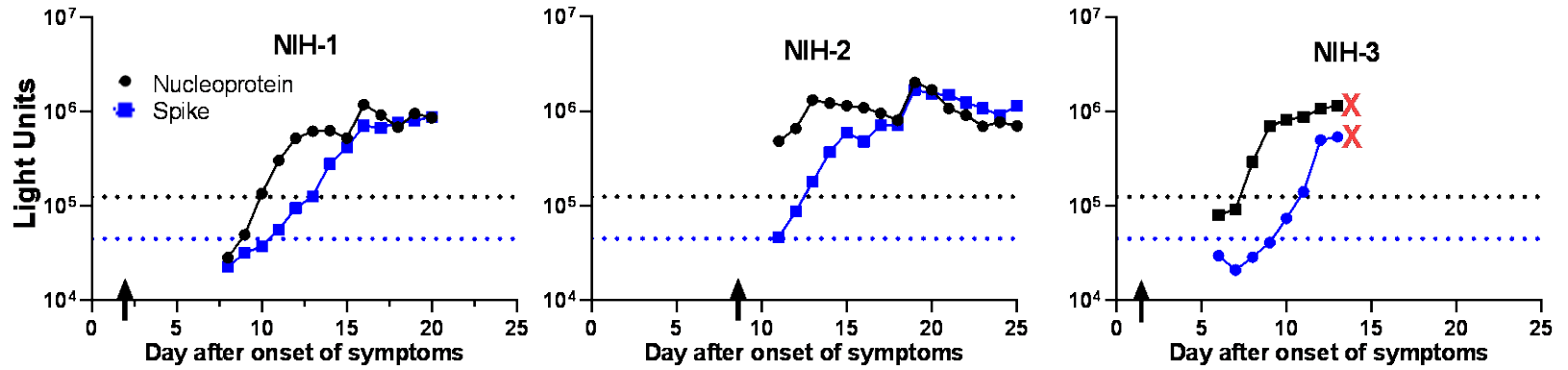[§]2 PCR negative and 8 not determined

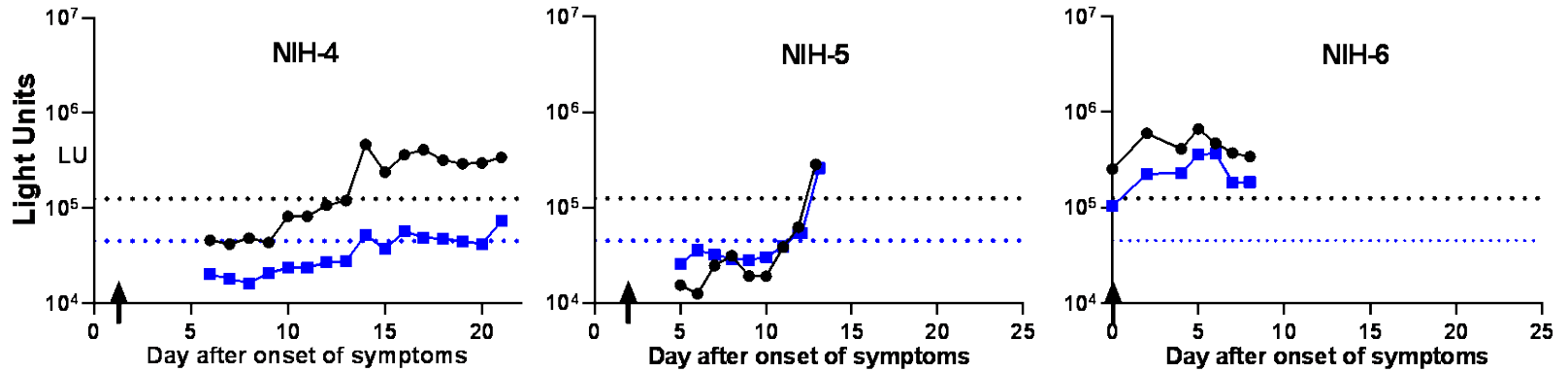[‡]Unknown for 1 subject

**Fig. 1**

# Fig. 2

**A.**



**B.**

**Fig. 3**



Plot axes: Unheated sample (y-axis) vs Heated sample (x-axis), both in LU. Correlation: Rs=0.92 (p<0.0001).