

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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23

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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

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

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 -80° 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 56° 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- $\Delta$ 2 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<sub>2</sub>, 1% Triton X-100) and 10  $\mu$ l 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**



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  $\leq 14$  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  $\leq 14$  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.

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° 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 ( $R_s=0.913$ ;  $P<0.0001$ ). 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

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  $\geq 14$  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

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 56°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 56°C 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.

340

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345

346



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417

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<sub>10</sub> 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<sub>10</sub> 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.

441

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 ( $R_s$ ) 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**

	<b>N</b>	<b>Gender (M:F)</b>	<b>Age years</b>	<b>With one or More Risk Factors*</b>	<b>SARS-CoV-2 PCR Positive</b>	<b>Time from Symptoms to First Blood Draw</b>	<b>Ventilator</b>	<b>Mortality</b>
		<b>ratio</b>	<b>(range)</b>	<b>no. (%)</b>	<b>no. (%)</b>	<b>Average (range)</b>	<b>no. (%)</b>	<b>no. (%)</b>
Blood Donors	32	ND	ND	ND	ND	ND	ND	ND
Suspected Cases <sup>#</sup>	10	4:6	32(7-49)	0 (0) <sup>‡</sup>	0 (0) <sup>§</sup>	47.1 Days (26-79) <sup>‡</sup>	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) <sup>‡</sup>	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

<sup>#</sup>EvergreenHealth

<sup>§</sup>2 PCR negative and 8 not determined

<sup>‡</sup>Unknown for 1 subject

Fig. 1

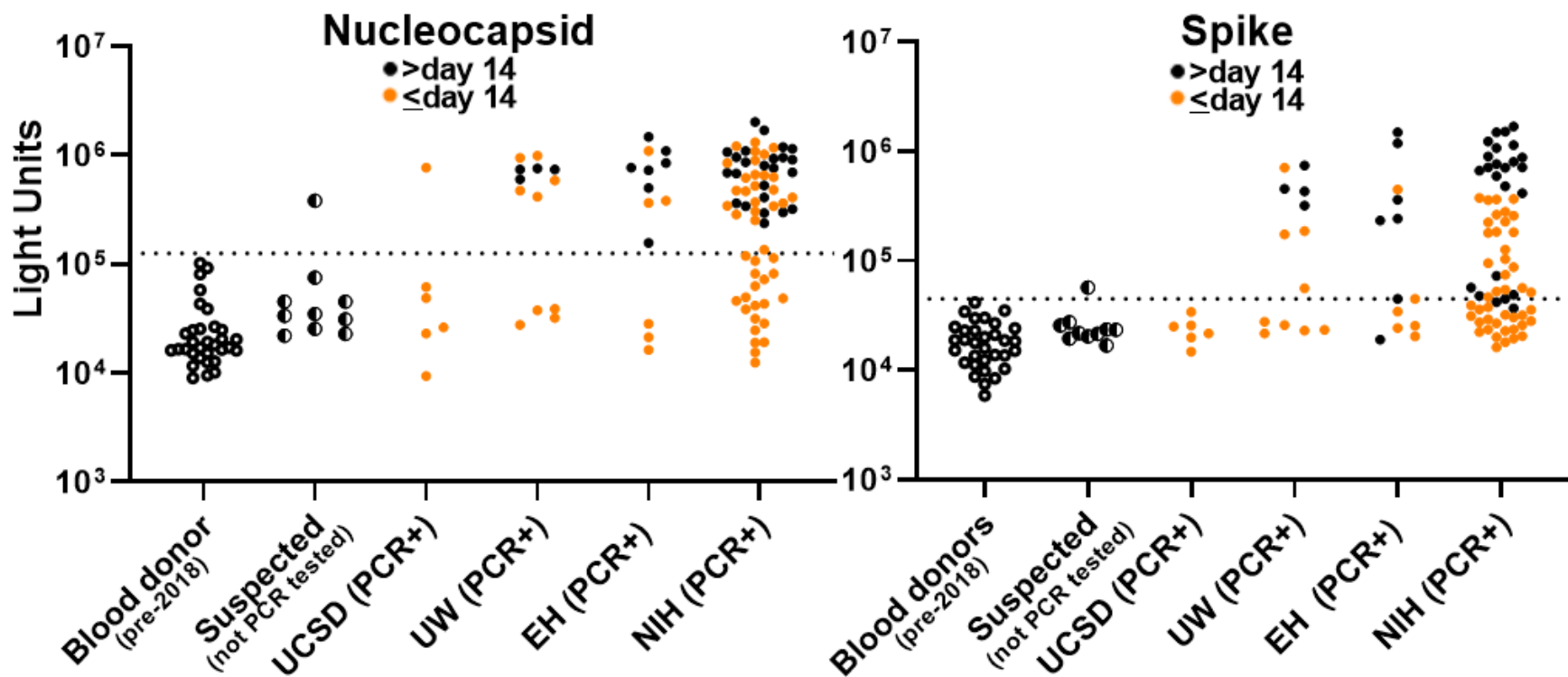
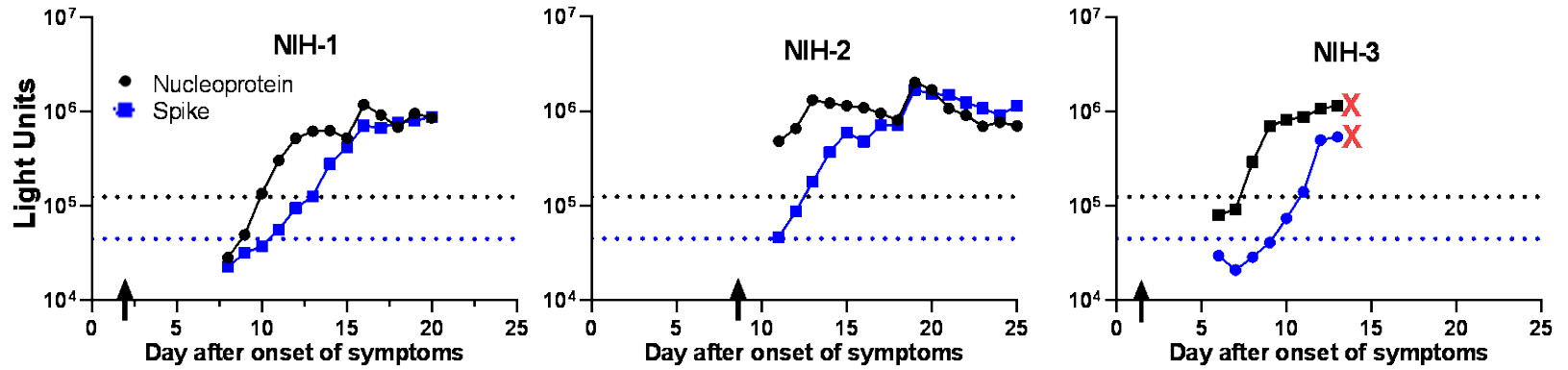


Fig. 2

A.



B.

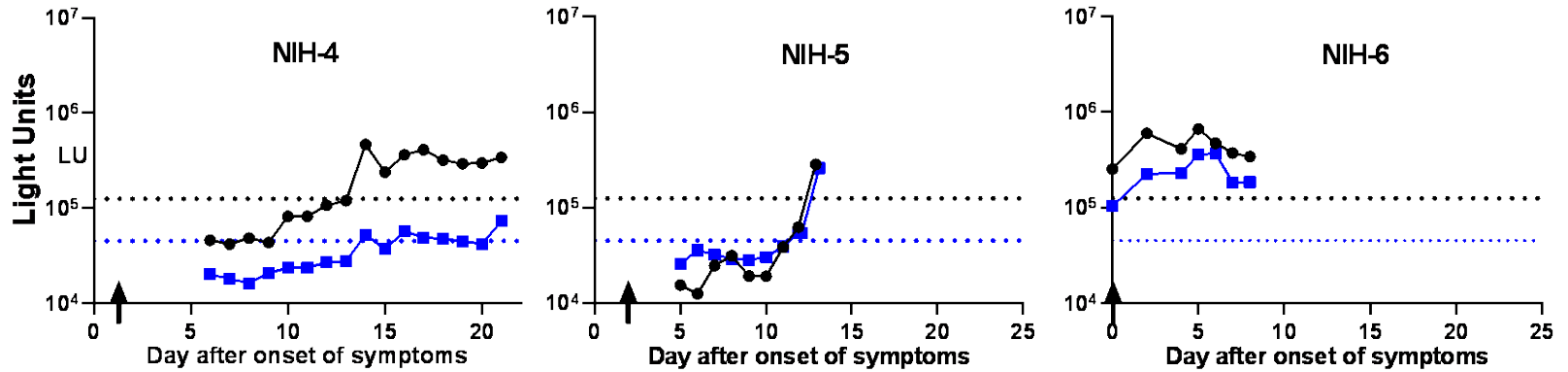


Fig. 3

