

Detection of Nucleocapsid Antibody to SARS-CoV-2 is More Sensitive than Antibody to Spike Protein in COVID-19 Patients

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Summary: An immunoprecipitation assay detected antibody to SARS-CoV-2 nucleocapsid protein with high sensitivity and specificity even after heat-inactivation of plasma. This assay was more sensitive than detection of antibody to the spike protein.

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Footnotes

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ABSTRACT

Background: SARS-CoV-2, the cause of coronavirus disease 2019 (COVID-19), is associated with respiratory-related morbidity and mortality. Assays to detect virus-specific antibodies are important to understand the prevalence of infection and the course of the immune response.

Methodology: Quantitative measurements of plasma or serum antibodies by luciferase immunoprecipitation assay systems (LIPS) to the nucleocapsid and spike proteins were analyzed in 100 cross-sectional or longitudinal samples from SARS-CoV-2-infected patients. A subset of samples was tested with and without heat inactivation.

Results: Fifteen or more days after symptom onset, antibodies against SARS-CoV-2 nucleocapsid protein showed 100% sensitivity and 100% specificity, while antibodies to spike protein were detected with 91% sensitivity and 100% specificity. Neither antibody levels nor the rate of seropositivity were significantly reduced by heat inactivation of samples. Analysis of daily samples from six patients with COVID-19 showed anti-nucleocapsid and spike antibodies appearing between day 8 and day 14 after initial symptoms. Immunocompromised patients generally had a delayed antibody response to SARS-CoV-2 compared to immunocompetent patients.

Conclusions: Antibody to the nucleocapsid protein of SARS-CoV-2 is more sensitive than spike protein antibody for detecting early infection. Analyzing heat-inactivated samples by LIPS is a safe and sensitive method for detecting SARS-CoV-2 antibodies.

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

Infections with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing coronavirus disease 2019 (COVID-19), were first reported in China [1-4]. The major clinical feature of SARS-CoV-2 infection is virus-associated pneumonitis [5-7]. In comparison to highly pathogenic coronaviruses such as SARS-CoV-1 and Middle East respiratory syndrome coronavirus (MERS-CoV) [8], SARS-CoV-2 spreads more rapidly and reached six of the seven continents, including North America [9], within three months of the initial outbreak. Nucleic acid-based testing of oropharyngeal or nasopharyngeal swabs and saliva is useful for diagnosing acute infection. SARS-CoV-2 virus RNA can often be detected in upper respiratory secretions at the time of the first appearance of symptoms, peaks during the first week, and declines with time [10, 11]. RNA from SARS-CoV-2, like the related SARS-CoV-1 [12], can be detected in blood [11, 13], and high levels of circulating viral RNA are associated with more severe disease [13].

Assessment of the antibody response to SARS-CoV-2 should complement the RNA-based tests and improve our understanding of the pathogenesis and course of COVID-19, contribute to epidemiological studies and inform vaccine development. Antibodies to the nucleocapsid protein are the most sensitive target for serologic diagnosis of infection with SARS-CoV-1 [14, 15]. Antibodies against the spike protein of SARS-CoV-1, the target of neutralizing antibody and vaccine development, emerge at a later time than those against the nucleocapsid protein. Recently, several groups have reported serological tests using the nucleocapsid and/or spike protein from SARS-CoV-2 by ELISA [11, 16, 17], immunofluorescence [18] and even a lateral flow test [19]. One study using ELISA to measure only antibodies to the nucleocapsid protein found that patients become seropositive 10-18 days after the onset of symptoms [16]. A commercial ELISA using the spike protein demonstrated that IgG antibodies were detectable at a median of 14 days after onset of symptoms [17]. To et al. examined antibodies against both the spike and nucleocapsid by

ELISA in a small number of samples and found that IgG antibodies against the nucleocapsid protein were generally detectable at about the same time as antibodies to the spike protein [11]. Despite these findings, further studies are needed to understand antibody dynamics in persons infected with SARS-CoV-2 to determine the most sensitive and specific antibody assays, and to use these antibody-based tests to determine seroprevalence in different populations. In addition, it is currently unknown whether the viral RNA that has been detected in the blood [11, 13] indicates the presence of infectious virus, which has the potential to be a safety hazard for clinical laboratory technicians and researchers analyzing serology in persons infected with SARS-CoV-2. Thus, a sensitive and specific antibody assay using heat-treated plasma/serum may enhance safety when working with these fluids.

We and others have employed a liquid phase immunoassay technology termed Luciferase Immunoprecipitation Systems (LIPS) to measure antibodies against many different viruses, to stratify infected patients based on antibody levels, and for virus discovery [20]. LIPS has shown promise for detecting antibodies against coronaviruses including the nucleocapsid of MERS-CoV [21] and the spike protein of swine acute diarrhea syndrome coronavirus (SADS-CoV) [22]. Unlike ELISA, which is solid phase, LIPS is performed in solution, thus maintaining the native antigen conformation. The antigen is produced in mammalian cells and often retains post-translational modifications of the antigen, unlike bacterial recombinant proteins or peptide based ELISAs. LIPS assays typically have a dynamic range up to 6 log₁₀ for some antigens and require < 5 ul of plasma or sera for testing. Here recombinant nucleocapsid and spike protein from SARS-CoV-2 as antigens in LIPS assays were used to measure antibodies in patients with COVID-19 from four United States locations. The LIPS assay showed high sensitivity and specificity for detecting SARS-CoV-2 antibodies and demonstrated that nucleocapsid antibodies emerge before spike antibodies. Moreover, as there are potential safety issues related to the presence of SARS-CoV-2 RNA in

blood, we show that heat inactivation of plasma at 56°C for 30 min does not significantly reduce the sensitivity of the LIPS assay and allows testing to be performed more safely.

METHODS

Characteristics of the COVID-19 patients

This retrospective study analyzed both cross-sectional and longitudinal blood samples collected from patients with COVID-19 or controls from four sites. Anonymized plasma or serum from patients from University of California, San Diego (UCSD, n=3), University of Washington, Seattle (UW, n=17), EvergreenHealth, Kirkland, Washington (EH, n=23) (**Table 1**) were obtained under an IRB exemption. Plasma from patients at the NIH Clinical Center, NIH (n=6) were obtained under a protocol approved by the IRB of the NIH Intramural Research Program; all patients signed consent. Anonymized blood bank donor controls (n=32) collected at the NIH Clinical Center prior to 2018 were used as uninfected controls. The time interval between the initial symptoms and obtaining plasma/serum samples from the 35 PCR+ confirmed cases was variable and ranged from 2 to 50 days. SARS-CoV2 infection was confirmed in each case by reverse transcriptase PCR detection of viral RNA from oropharyngeal or nasopharyngeal swabs performed at clinical laboratories from each location. For the NIH samples, serial daily blood drawn samples (n=68) were available covering 0-20 days from symptom onset.

Storage and Heat Inactivation

Plasma/serum samples were collected and stored frozen at -80° C, except for the heat-inactivated samples from the NIH that were not previously frozen. In light of previous studies that demonstrated a marked loss in infectivity of SARS-CoV-1 [23] and MERS [24] coronaviruses with heating, we adopted a precautionary safety protocol performed before

analysis. Aliquots of plasma/serum from each patient sample were first incubated at 56° C for 30 min and then used for testing as described below.

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

Antibodies

LIPS assays, in which viral proteins fused to light-emitting luciferase are immunoprecipitated, were essentially performed as described [25]. The SARS-CoV-2 nucleocapsid was constructed in the standard pREN2 vector as a C-terminal *Renilla* luciferase fusion protein. The spike protein, due to its signal peptide sequence, was generated as an N-terminal fusion with a different luciferase, *Gaussia* luciferase, in the pGAUS3 vector for better expression of the fusion protein [26]. The nucleocapsid sequence of SARS-CoV-2 (GenBank MN908947) was generated as a synthetic DNA (Twist Biosciences) and then cloned into the pREN2. A spike protein construct of SARS-CoV-2 (amino acids 1-538 of GenBank MN908947) was generated by PCR from a plasmid containing a prefusion form of the spike protein (2019-nCoV-2_S-2P) [27] and subcloned to generate pGAUS3-Spike. A second spike construct, pGAUS3-Spike-Δ2 (amino acids 1-513) was constructed in the pGAUS3 vector in the same way. Preliminary tests comparing antibody detection using pGAUS3-Spike-Δ2 and pGAUS3-Spike showed similar results and the former construct was not used further.

Nucleocapsid and spike protein-light emitting plasmid constructs were transfected into Cos1 cells and lysates harvested 48 hours later to obtain crude cell extracts. For testing, heat-inactivated serum/plasma samples were diluted 1:10 in assay buffer A (20 mM Tris, pH 7.5, 150 mM NaCl, 5 mM MgCl₂, 1% Triton X-100) and 10 µl of the diluted sample were then tested in a 96-well microtiter plate as described [25]. After incubation for one hour, the mixture was transferred to a microtiter filter plate containing protein A/G beads and

incubated for one hour. Protein A/G beads efficiently bind IgG and to a much lesser extent IgM, to capture the antibody-antigen-bead complexes. Microtiter plates containing the beads were then washed eight times with buffer A and twice with PBS to remove unbound antigens. After the final wash, coelenterazine substrate (Promega) was added to detect the luciferase activity and light units (LU) were measured in a Berthold LB 960 Centro microplate luminometer (Berthold Technologies, Bad Wildbad).

Percentages for categorical variables, mean and range, geometric mean plus 95% CI were used to describe some of the data. Antibody levels were reported as the geometric mean level (GML) with 95% confidence interval (CI). Cut-off limits for determining positive antibodies in the SARS-CoV-2-infected samples were based on the mean plus three standard deviations of the serum values derived from the 32 uninfected blood donor controls or by receiver operator characteristics (ROC) analysis. Based on the typical two-week period between when a virus is initially encountered and the time to generate virus-specific IgG antibody, time intervals of $>$ or ≤ 14 days was chosen for calculating sensitivity of the LIPS assays. Wilcoxon signed rank and Fischer's exact test were used for statistical analysis.

RESULTS

Characteristics of the patients with COVID-19

Patients with COVID-19 were located in four geographically distinct locations across the United States and included 35 SARS-CoV-2 cases confirmed by PCR, 10 subjects with COVID-19-like symptoms or household contacts of persons with COVID-19 (not tested by PCR), and 32 blood donors who donated samples before 2018 were used as controls (Table 1). The majority of the SARS-CoV-2 PCR-confirmed cases were male (87%) and the median age was 44 years (range, 32-50 years). A subset of the SARS-CoV-2 PCR-confirmed cases

had one or more risk factors including heart disease, lung disease, diabetes, and/or they were immunocompromised. Two different plasma samples, drawn 2-3 days apart, were available for each of the three COVID-19 cases from the UCSD and multiple daily samples were available from the NIH patients with COVID-19. Combining the cross-sectional and longitudinal studies resulted in 100 samples from PCR+ patients.

Detection of Antibodies to the Nucleocapsid Protein is More Sensitive than Antibodies to the Spike Protein in COVID-19 Patients

LIPS assays for detecting antibodies were developed using SARS-CoV-2 nucleocapsid and spike antigens produced in mammalian cells. Pilot experiments using nucleocapsid-*Renilla* luciferase and spike protein-*Gaussia* luciferase fusion proteins were conducted with serum/plasma from blood donor controls collected prior to 2018. Results showed a low background with little or no antibody immunoreactivity against the spike protein, but there was a higher background against the nucleocapsid (data not shown). The specificity of the two SARS-CoV-2 LIPS assays was also tested with four serum samples from patients that had recently tested positive by PCR of oropharyngeal or nasopharyngeal samples for other coronaviruses. Serum samples from two different patients infected with the HKU1 beta-coronavirus and two different patients infected with the NL63 alpha-coronavirus were seronegative by LIPS for SARS-CoV-2 nucleocapsid and spike antibodies (data not shown). In addition, using our previous LIPS test for MERS nucleocapsid protein [21] which has higher amino acid homology with the SARS-CoV-2 nucleocapsid than the seasonal coronaviruses [28], we found no seropositivity for the MERS nucleocapsid protein in COVID-19 positive patients, highlighting the specificity of the SARS-CoV-2 LIPS assays (data not shown). In order to develop highly specific serological tests without potential false positives, stringent cut-off values from the blood donor controls were assigned based on

statistical methods and/or ROC. Cut-off values for the nucleocapsid and spike proteins were derived from the mean plus four standard deviations (125,000 LU) and the mean plus three standard deviations (45,000 LU) of the blood donor controls, respectively.

Using these cut-off values, plasma/serum samples from the COVID-19 cohort were evaluated by the two LIPS assays. For safety reasons, all samples were heated at 56°C for 30 min to reduce the likelihood of infectious virus in the samples. Coded samples from suspected COVID-19 cases from EH were then tested as well as noncoded pre-2018 blood donors, and SARS-CoV-2 PCR-positive cases from the UCSD, UW, EH, and the NIH Clinical Center (NIH). A wide dynamic range of antibody levels against the nucleocapsid and spike protein were observed differing by up to 100-fold between samples (**Figure 1**). In order to compare the sensitivity of the nucleocapsid and spike LIPS assays, a minimum interval of >14 days between onset of symptoms and time of blood collection was used to determine the number of seropositive serum or plasma samples in the SARS-CoV-2 PCR-positive group. Among the PCR+ patient samples collected >14 days after onset of symptoms (**Figure 1, black dots**), seropositive nucleocapsid antibodies were detected in 35/35 samples yielding both a sensitivity and specificity of 100%. A similar analysis of the spike antibody in samples collected >14 days after onset of symptoms showed a slightly lower sensitivity of 91% (32/35) with 100% specificity, but this was not statistically different than nucleocapsid (Fischer's Exact test). Comparison of antibody levels of nucleocapsid with spike antibodies revealed that they tracked with each other well ($R_s=0.72$, $p<0.0001$).

Evaluation of samples collected at ≤ 14 days after onset of symptoms showed reduced sensitivity, but specificity was maintained. The sensitivity for antibody to the nucleocapsid protein at this time point was 51% (33/65) (**Figure 1, orange dots**). Analysis of spike antibodies of samples collected at ≤ 14 days after onset of symptoms showed a sensitivity of 43% (28/65). Taken together, our findings indicate that detection of antibodies against the

nucleocapsid protein is more sensitive than detection of antibodies against the spike protein, and that nucleocapsid antibodies generally appear earlier than spike antibodies.

In addition to the SARS-CoV-2 PCR-confirmed patients, suspected cases of COVID-19 from EH were also analyzed for seropositivity. Nine of the ten suspected cases without viral PCR confirmation, with some showing symptoms of fever, cough and/or fatigue compatible with COVID-19 and others with household exposure to COVID-19 cases, collected between January and February 2020, were seronegative for both nucleocapsid and spike antibodies (**Figure 1**). Interestingly, one case from March 2020 from a person who was a household contact with a SARS-CoV-2 PCR+ patient, was seropositive for both nucleocapsid and spike antibodies.

Since there is interest in using serological assays to assess current and historical infections, we evaluated the robustness of the LIPS assay for detecting SARS-CoV-2 antibodies by analyzing the level of antibodies in all PCR-confirmed samples collected more than 14 days after symptom onset. The geometric mean level (GML) of nucleocapsid antibody levels in the 35 seropositive samples was 694,600 (95% CI, 570,000-844,600 LU), which was ~32 times higher than the GML of the blood donor controls of 21,356 LU (95% CI, 17,032-26,752). Antibodies against spike protein showed a similar discriminatory potential for the seropositive samples with GML of 346,800 LU (95% CI, 218,800-550,000 LU), which was ~21 times higher than the blood donor controls with 16,843 LU (95% CI, 14,172-20,007 LU).

Time course of the appearance of antibodies against SARS-CoV-2 differs in immunocompetent and immunocompromised patients

To understand the timing and trajectory of SARS-CoV-2 antibodies against nucleocapsid and spike proteins, serial daily blood samples from the six NIH patients with COVID-19 were studied. In all six subjects, SARS-CoV-2 antibody levels rose with time in both the three immunocompetent (**Figure 2A**, NIH patients 1-3) and three immunocompromised patients (**Figure 2B**, NIH patients 4-6). These latter three patients had chronic lymphocytic leukemia, metastatic chordoma, or had received a hematopoietic stem-cell transplant. All three immunocompetent COVID-19 patients showed a rapid rise in antibody to nucleocapsid and began within 10 days of symptom onset in 2 patients (no samples were available before day 11 for the third patient, **Figure 2A**). Antibodies against the spike protein in these three immunocompetent patients generally tracked the nucleocapsid antibodies, but in one case seropositivity appeared 2 days later than nucleocapsid antibody. The third patient, NIH-3, with a history of hypertension and heart disease died of cardiovascular shock and hypoxemia 13 days after onset of symptoms.

Antibody profiles in three immunocompromised NIH patients showed more blunted responses against the SARS-CoV-2 antigens (**Figure 2B**). Patient NIH-4 became seropositive for both nucleocapsid and spike antibodies on day 14 and these antibodies then plateaued at these low levels for the next seven days. Similarly, patient NIH-5 did not become seropositive until day 13 for spike antibody and day 14 for nucleocapsid antibody. Patient NIH-6 was both PCR+ for SARS-CoV-2 and seropositive on the day of symptoms, suggesting that he had an asymptomatic infection for several days before diagnosis. Review of the non-NIH patients showed that of the five immunocompromised patients in this group, one had low levels of spike antibody and two were negative for spike antibody, but positive for nucleocapsid antibody.

Heat inactivation of plasma minimally impacts detection of SARS-CoV-2 antibodies in LIPS assays

While heating plasma to 56° C for 30 min has been shown to reduce the titer of coronaviruses, heating might reduce or eliminate IgM and IgG responses [29]. Therefore, we performed LIPS assays on a subset of the patients with COVID-19 from the known or suspected cases (N=38) with and without heat inactivation, to evaluate its impact on nucleocapsid antibody levels and seropositivity status. Evaluation of antibody responses in heated versus unheated plasma samples showed that antibody levels were mostly unchanged (**Figure 3**). In a single sample from one patient with COVID-19, antibody to SARS-CoV-2 was not detected after heat-inactivation. Of note, this sample came from an NIH patient with COVID-19 who was antibody positive at day 7 using non-heated plasma and became seropositive using heat-inactivated plasma from day 8. Statistical analysis showed no significant difference in antibody levels between plasma that was heated or unheated (Wilcoxon Signed rank test) and the values were highly correlative ($R_s=0.913$; $P<0.0001$). These findings indicate that the heat-inactivation process is diagnostically suitable for LIPS testing of SARS-CoV-2 antibodies.

DISCUSSION

The fluid-phase LIPS assay was used to investigate antibodies to the SARS-CoV-2 nucleocapsid and spike protein in COVID-19 patients after infection. The LIPS assay demonstrated high sensitivity and a wider dynamic range for antibody detection compared to other published assays [11, 16-19]. Analysis of longitudinal plasma samples showed that antibodies against the nucleocapsid and spike proteins appeared about the same time between day 8 and day14 after the onset of symptoms. Only one study to date has examined antibodies separately against the nucleocapsid protein and spike protein [11] and our findings

are in general agreement. COVID-19 patient plasma samples obtained ≥ 14 days after symptom onset showed that the LIPS assay for antibodies against the nucleocapsid and spike protein had 100% and 94% sensitivity, respectively, with 100% specificity for both antibodies. Additional studies using this highly quantitative LIPS assay may help determine whether the relative levels of antibodies observed in convalescent COVID-19 patients or uninfected vaccinated persons correlate with prevention of reinfection or primary infection, respectively. Following humoral response profiles over time from convalescent COVID-19 cases should provide important insights into the half-life of these antibodies.

Our studies with serial patient samples from the NIH cohort showed the temporal relationship between antibody dynamics with onset of symptoms and PCR positivity for SARS-CoV-2. Cut-off values for a positive result was based on pre-2018 blood donors and may underestimate the number of seropositive persons because some individual patients showed low antibody values initially that gradually rose before exceeding the cut-off value. The three immunocompetent COVID-19 patients showed rapid seroconversion within 10 days of onset of symptoms for nucleocapsid antibody, and then spike antibodies appeared a day or so later. In contrast, the immunocompromised NIH patients exhibited a slower rise in antibody levels with a plateau at lower levels compared to the immunocompetent patients, and two patients did not become seropositive until 14 days after onset of symptoms. Despite the blunted antibody responses in the immunocompromised patients, they had a favorable clinical outcome. The NIH patient who died (NIH-3) was not immunocompromised and had a rapid rise in antibody production reaching levels comparable to the other immunocompetent patients. In addition, one of the two EH patients who died showed the highest antibody levels in that cohort of patients. While excessive proinflammatory responses to the virus have been reported to contribute to poor outcomes [30-32], larger studies of COVID-19 patients are required to determine whether antibody levels directly correlate with disease severity.

Prior studies have shown high levels of SARS-CoV-2 RNA in blood from some patients with COVID-19 [11, 13, 33]. At present, it is not certain whether infectious virus might be circulating in the blood early during infection. Accordingly, plasma or serum was heated to 56° C for 30 min to reduce the titer of SARS-CoV-2 before performing the LIPS assays, since prior studies have shown a marked loss in infectivity of SARS-CoV-1 [23, 34] and MERS [24] coronaviruses with heat treatment. While impaired detection of viral IgM and IgG antibody responses to viruses after heating samples to 56°C has been reported [29], and several abstracts report similar findings with SARS-CoV-2 samples, our direct comparison of untreated and treated samples found high concordance of the antibody values revealing the suitability of heat inactivation. Serological studies with other antigens have shown that the activity of IgM is markedly reduced or destroyed by 56°C heating, while IgG is often preserved [29, 35]. Thus, the lack of difference in antibody levels we observed after heating to 56°C is suggestive that the antibodies measured were primarily IgG.

A limitation of our study was the focus on more severe COVID-19 cases rather than asymptomatic cases for assay validation. Asymptomatic cases may show lower antibody responses. Further modification of LIPS assays for detection of SARS-CoV-2 antibodies, including the use of other immunoglobulin capture reagents (e.g. anti-IgA, -IgG or -IgM beads) for measuring isotype-specific antibodies, different SARS-CoV-2 protein fragments, or different luciferase reporters, may further expand or improve assay performance. Nonetheless, our current assay provides quantitative results with a high degree of sensitivity and specificity and should be useful for larger seroepidemiologic studies.

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Figure legends

Figure 1. Detection of antibodies against SARS-CoV-2 nucleocapsid and spike protein in patients with COVID-19. Antibody levels against SARS-CoV-2 nucleocapsid and spike protein were determined in 32 pre-2018 blood donors, 10 suspected COVID-19 cases (not PCR confirmed) from EvergreenHealth, Kirkland, WA (EH), three PCR+ COVID-19 patients from UCSD, 13 PCR+ COVID-19 patients from the University of Washington (UW), 13 PCR+ COVID-19 patients from EH, and 6 COVID-19 patients from the NIH Clinical Center (NIH). Each symbol represents a sample from an individual patient or different time points from an individual patient. Antibody levels are plotted in light units (LU) on a $\log_{10}$ scale. Black circles represent plasma or serum samples obtained after 15 or more days after symptom onset and orange circles are from plasma or serum samples obtained 14 or less days after symptom onset. The dashed lines represent the cutoff level for determining positive antibody titers as described in the Methods.

Figure 2. Longitudinal profile of antibodies against nucleocapsid and spike protein in immunocompetent and immunocompromised COVID-19 patients from NIH. Antibody levels were determined in daily blood draws from six COVID-19 patients. Three of the COVID-19 patients were immunocompetent (**Panel A, NIH-1-3**) and three (**Panel B, NIH-4-6**) were immunocompromised. The levels of antibody to the nucleocapsid (black line) and spike protein (blue line) over time are shown and were plotted on the y-axis using a $\log_{10}$ scale. Time zero represents the first day symptoms appeared, and the vertical arrows are the time of diagnosis by PCR. The cut-off values for determining seropositivity is shown by the dotted lines. The red X's indicates the day after onset of symptoms that patient NIH-3 died.

Figure 3. Heat inactivation of plasma or serum samples has no significant impact on detection of nucleocapsid antibodies. A subset (n=38) of plasma samples from patients with COVID-19 including samples from PCR-positive patients from very early infection (less than 8 days) and at later times after initial infection were analyzed. Levels of antibody to the nucleocapsid protein were determined by LIPS for aliquots of paired samples from unheated plasma or serum and from heated plasma or serum. Antibody levels were plotted, and the horizontal and vertical dotted lines represent the cutoff values for seropositivity. The diagonal line is a theoretical value if the antibody levels were identical for heated and unheated samples. The antibody values strongly correlated for heat treated and not heat-treated samples as shown by the Spearman rank correlation (R_s) of 0.92 ($P < 0.0001$) and only one sample showed a significant decrease with heating.

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Table 1. Subject Characteristics of COVID-19 Cohort

	N	Gender (M:F)	Age years	With one or More Risk Factors*	SARS-CoV-2 PCR Positive	Time from Symptoms to First Blood Draw	Ventilator	Mortality
		ratio	(range)	no. (%)	no. (%)	Average (range)	no. (%)	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. 0 of 3 patients from Univ. Calif., San Diego, 4 of 13 from Univ. of Washington, 1 of 13 from EvergreenHealth, and 3 of 6 from NIH were immunocompromised.

Abbreviation: ND, not determined

[#]EvergreenHealth, Kirkland, WA

[§]2 PCR negative and 8 not determined

[‡]Unknown for 1 subject

Figure 1

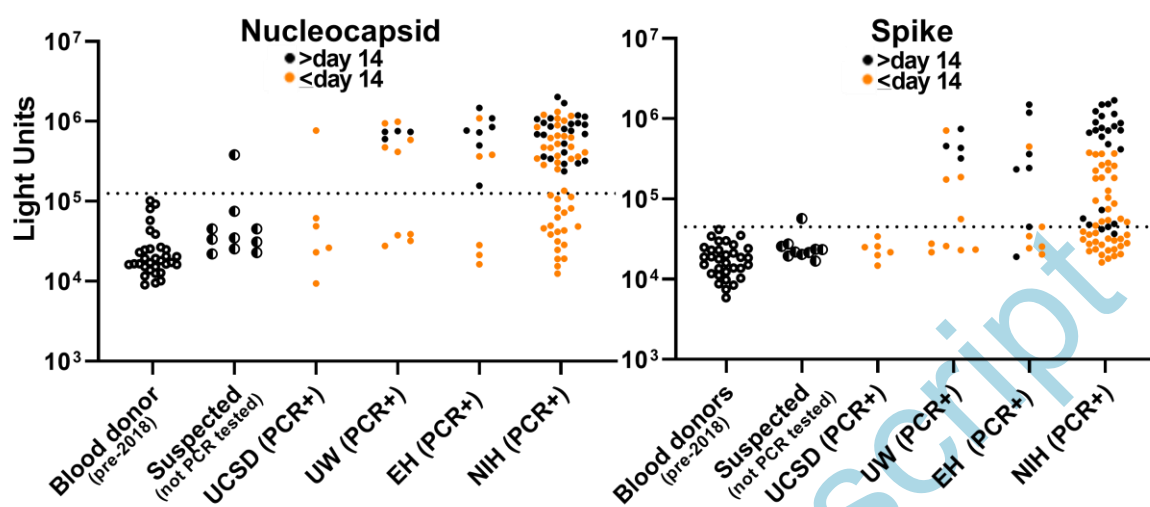


Figure 2

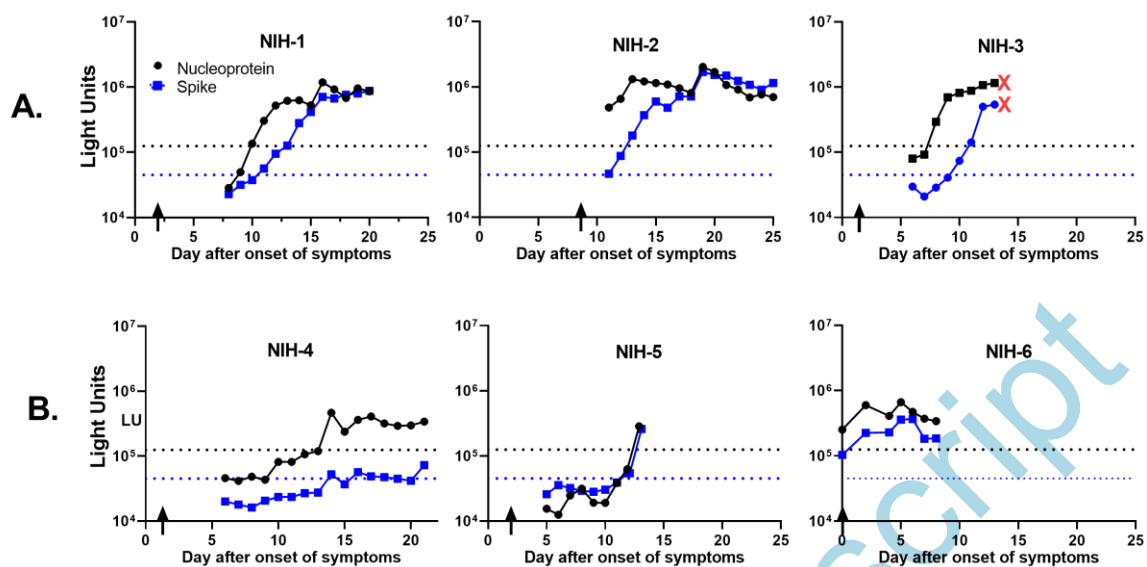


Figure 3

