1 **Humoral immune response and prolonged PCR positivity in a cohort of 1343**

2 **SARS-CoV 2 patients in the New York City region**

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12 **Abstract**

13 *Background:* Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a

14 global pandemic. The percentage of infected individuals who seroconvert is still an open

15 question. In addition, it has been shown in some individuals that viral genome can still be

16 detected at considerable time post symptom resolution. Here we investigated both

17 seroconversion and PCR-positivity in a large cohort of convalescent serum donors in New York

18 City.

19 *Methods:* Individuals with confirmed or suspected SARS-CoV-2 infection were screened via

20 PCR for presence of viral genome and via enzyme-linked immunosorbent assay for presence of

21 anti SARS-CoV-2 spike antibodies.

22 *Results:* All but three confirmed SARS-CoV-2 patients seroconverted to the SARS-CoV-2 spike

23 while only 37.4% of suspected SARS-CoV-2 patients seroconverted. PCR-positivity was

24 detected up to 28 days from symptom resolution.

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25   *Conclusions:* Here we show that the vast majority of confirmed COVID19 patients seroconvert,

26   potentially providing immunity to reinfection. We also report that in a large proportion of

27   individuals, viral genome can be detected via PCR in the upper respiratory tract for weeks post

28   symptom resolution, but it is unclear if this signal represents infectious virus.

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32   **Introduction**

33   Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has rapidly spread around the

34   world, leading to unprecedented strain on health care systems and economies, causing over

35   290,000 infections and 17,000 deaths in New York State (source, New York State, Department

36   of Health as of April 28th 2020) at the time of writing this report. Significant disruptions to daily

37   life have been enacted in order to flatten the epidemic curve. In order to avoid spread of SARS-

38   CoV-2 and to help standardize definitions of clearance, it is important to understand the duration

39   of SARS-CoV-2 nucleic acids within the nasopharynx and the time course to the mounting of an

40   antibody response to this new viral pathogen.

41   Current US Centers for Disease Control and Prevention (CDC) guidelines suggest that people

42   with confirmed or suspected SARS-CoV-2 infection should remain in isolation for at least seven

43   days from symptom onset and return to work if they have been asymptomatic for 72 hours.

44   However, to date, there are limited data that help to define the time to viral clearance from illness

45   onset and cessation of symptoms. A prior case study suggested that SARS-CoV-2 can exhibit

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46   ongoing viral shedding for a median of 2.5 days (range 1-8 days) after complete symptom

47   resolution, but it remains unclear whether this viral shedding poses a risk for forward

48   transmission.[1] A small case sample of four recently hospitalized patients found that they were

49   SARS-CoV-2 positive on repeat polymerase chain reaction (PCR) testing five to 13 days after

50   discharge.[2] Other studies have found viral shedding for up to six weeks after symptoms.[3] From

51   work with the 2003 SARS-CoV-1it is not clear if detection of viral genome of this duration

52   indicates prolonged infectivity or presence of nonviable virus.[4] A clearer understanding of

53   duration of viral shedding is critical to preventing transmission by infected individuals,

54   particularly as they begin to feel well enough to resume normal activities. Understanding time to

55   PCR clearance may also help guide isolation durations and return to work clearance, as well as

56   clarify the utility of negative PCR testing as part of defining disease clearance.

57   There is limited data worldwide on the development of antibodies to SARS-CoV-2, particularly

58   the formation of IgG. While there is concern regarding efficacy of antibody testing for diagnosis

59   of SARS-CoV-2, little is known about long lasting immunity. One study measured neutralizing

60   antibodies in 175 hospitalized patients and found that 64% had high antibody titers, 30% had

61   weak antibody response, and 6% had undetectable titers.[5] Studying the plasma from previously

62   infected individuals may further our understanding of the timing and strength of different

63   populations' antibody response to this novel illness, delineate duration of antibody presence, and

64   identify cases of possible reinfection. Additionally, individuals with high antibody titers may

65   become donors for convalescent plasma treatment for critically ill patients as part of ongoing

66   studies of this therapeutic option.[6-8]

67   Here we present a large data set of serum antibody testing in persons who have fully recovered

68   after mild illness from SARS-CoV-2 at Mount Sinai Hospital. Our aim is to describe the time to

69   SARS-CoV-2 PCR clearance from the nasopharynx, the rates of IgG development, and time to

70   serum IgG development from onset and resolution of symptoms in 1,343 participants with prior

71   confirmed or suspected SARS-CoV-2 infection.

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73   **Methods**

74   We conducted an outreach program in the New York City area, including parts of New York

75   State, Connecticut, and New Jersey, to identify people recovered from SARS-CoV-2 for

76   nasopharyngeal PCR (cobas® SARS-CoV-2, Roche Diagnostics, Indiana) and serum IgG titer

77   measurement (ELISA Mount Sinai).[9,10] Participants were tested between March 26 and April 10,

78   2020. We recruited participants via REDCap® (Vanderbilt University, Tennessee) online survey

79   response which was advertised on our hospital website, and subsequently shared by multiple

80   news organizations and public officials in New York. REDCap respondents were deemed

81   eligible if they had previously tested positive for SARS-CoV-2, or if they were symptomatic

82   with suspected SARS-CoV-2 and lived with someone with a positive SARS-CoV-2 PCR test,

83   had been told by a physician that they had symptoms consistent with SARS-CoV-2, or were

84   healthcare workers. We only included participants who self-reported suspicious symptoms after

85   February 1st 2020, as this is when it is believed SARS-CoV-2 began to spread in New York

86   City. Additionally, only participants who were asymptomatic at time of survey were

87   outreached. Respondents self-reported date of symptom onset, date of positive SARS-CoV-2

88   test (if applicable), and last date of symptoms. Duration of symptoms was calculated from these

89   self-reported dates.

90   During the first two weeks of the survey, we tested for SARS-CoV-2 in the nasopharynx by PCR

91   as well as IgG antibody (Ab) in the serum of every individual while in the third week the testing

92   was limited to SARS-CoV-2 antibodies only. The rationale for this change was that we had a far

93   larger testing volume combined with the fact that many of the participants reported symptom

94   resolution >14 days prior to testing. During week one, participants were brought in ten days after

95   they had a confirmed/suspected diagnosis and had been asymptomatic for at least three days. In

96   week 2, as we identified more potential donors and learned more about our antibody assay, we

97   extended our timeline to 14 days after symptoms onset, with at least three days asymptomatic. In

98   week 3, we included participants 21 days or more after symptom onset, who had been

99   completely asymptomatic for at least 14 days.

100   SARS-CoV-2 PCR was considered positive if detected on nasopharyngeal swab. We measured

101   serum IgG antibody titers using a serologic enzyme-linked immunosorbent assay (ELISA)

102   developed at Icahn School of Medicine at Mount Sinai and described on March 18, 2020; this

103   serum test has a sensitivity of 92% and a specificity >99%. Serum IgG titers were considered

104   "strongly positive" if they were detected at titers of 1:320 or higher (highest dilutions were

105   1:320, 1:960, 1:2880), and considered "weakly positive" if detected at titers of 1:80 and 1:160.

106   Negative was defined as titers below 1:80 (and is shown in figures as 1:40). All interested

107   participants with antibody titers >1:320 and negative SARS-CoV-2 PCR swabs were screened by

108   the New York Blood Center using standard criteria for plasma donation and included as donors

109   in our convalescent plasma study if eligible as per CFR Title 21. Participants with weakly

110   positive antibody titers were invited to return for repeat serum titer testing at least seven days

111   after their initial antibody test. Participants with positive PCR swabs and antibodies were asked

112   to return for PCR testing at least three days after initial PCR test so they could be referred for

113   plasma donation once the virus had fully cleared. Given the frequent serial detection of SARS-

114   CoV-2 by nasopharyngeal PCR using three-day increments, on April 3rd, 2020, we began to ask

115   participants to return at least ten days after the last positive PCR for re-testing.

116   One-way ANOVA and Fisher's exact test were used to measure the association of age, gender,

117   symptom duration, and days from symptom onset and resolution with positive antibody results.

118   This study was reviewed and approved by our institutional review board.

119   **Results**

120   We measured SARS-CoV-2 antibody titers in 1,343 people over the first three weeks of the

121   survey (March 26, 2020 to April 10, 2020) using a now FDA approved two step ELISA.[9,10] The

122   average age of the participants was 40 years (range 17 to 76 years) with 256 (19%) between the

123   ages of 17 and 29, 968 (72%) age 30-59, and 119 (9%) 60 or older. Fifty three percent were

124   male and 47% had confirmed SARS-CoV-2 diagnosis by prior PCR testing. Median days

125   between symptom onset to serum antibody test was 24 days (range 3 -70), median days between

126   symptom resolution to antibody test was 15 (4-77) and median duration of symptoms was 9 days

127   (1-67). Of the 1,343 total participants, almost all were outpatients who had experienced mild to

128   moderate symptoms, and only 3% were seen in our emergency department or hospital.

129   Seven hundred and sixty-one (57%) participants of the total sample were antibody positive, 61

130   (5%) were weakly positive, and 521 (39%) were negative. Neither age, gender, nor symptom

131   duration was associated with antibody response. In the 584 participants for whom both

132   nasopharyngeal PCR testing and serum antibody testing was available, SARS-CoV-2 RNA was

133   detected in 249 (42%) at a median of 20 days from symptom onset (range 11-43) and 12 days

134   from symptom resolution (range 5-28).

135   Six hundred and twenty-four participants had confirmed SARS-CoV-2 disease by PCR prior to

136   coming for testing, either by self-report or documented in our electronic medical record; if self-

137   reported, participants provided the date of testing. In this subgroup the average age was 39

138   (range 17-76) and 59% were male. At first test, five hundred and eleven (82%) were strongly

139   antibody positive at titer >= 1:320, 42 (7%) were weakly positive, and 71 (11%) were negative

140   (**Figure 1A**). We asked the 18% with initial negative or weakly positive antibody response to

141   return for a second test ten or more days later. At the time of the first test, 217 (35%) were still

142   PCR positive (range 5-22 days from symptom resolution) (**Figure 2**). Median duration of

143   symptoms in this group was nine days (range 1-31). Neither age nor sex was associated with a

144   strong antibody response. Greater number of days between symptom onset and antibody test

145   was associated with a higher titer antibody test (23 days versus 20 days). Symptom duration was

146   also associated with higher antibody titers (9 days versus 7 days). (**Table 2**)

147   In the subgroup of 719 participants with suspected disease that did not have confirmed SARS-

148   CoV-2 infection, average age was 41 (17-76) and 47% were male. 250 (35%) were strongly

149   antibody positive, 19 (3%) were weakly positive, and 436 (62%) were negative. Antibodies

150   were measured a median of 28 days from symptom-onset and 19 days from date of symptom

151   resolution. In this group, neither age, gender, nor symptom duration was associated with

152   stronger antibody response. (**Table 1**)

153   Of the 113 participants with PCR confirmed SARS-CoV-2 and weakly positive or negative titers

154   on their first serum antibody test, 64 have returned for follow up antibody titers at the time of

155   submission. Of these, 57 (89%) displayed increased titers between the two tests, a median of 13

156   days (5-25) later (**Figure 1B**). Four remained weakly positive, and three remained negative.

157   The three that remained negative all self-reported positive PCR testing (none were documented

158   in our EMR). (**Table 2**)

159   While all survey participants self-reported complete resolution of symptoms three to 14 or more

160   days prior to testing, 249 (19%) tested positive for nasopharyngeal SARS-CoV-2 RNA. The

161   maximum time of positive nasopharyngeal PCR testing was 43 days from symptom onset and 28

162   days from symptom resolution. For the 182 individuals who returned for repeat nasopharyngeal

163   swabbing at least three days after prior positive test, 61% were negative on the repeat test, a

164   median of 10 days (range 3-21) after the first test. Seventy (39%) remained positive and were

165   rescheduled for another nasopharyngeal PCR 7-10 days later. (**Table 3**).

166   **Discussion**

167   Our survey provides a large cross-sectional representation of SARS-CoV2 RNA and antibodies

168   found in participants recruited after recovery from SARS-CoV-2 during the early weeks of the

169   outbreak in New York City. Understanding the duration of potential infectiousness and the time

170   to IgG antibody response are critical to the containment of SARS-CoV-2 spread, and the plans

171   for widespread antibody testing over the coming months. Some countries, states and

172   organizations may even be considering antibody testing prior to letting individuals return to

173   work.

174   In contrast to some of the prior literature on formation of antibodies, over 99% of the patients

175   who self-reported or had laboratory documented SARS-CoV-2 infection developed IgG

176   antibodies using our assay. Additionally, our findings suggest that IgG antibodies develop over a

177   period of 7 to 50 days from symptom onset and 5 to 49 from symptom resolution, with a median

178   of 24 days from symptom onset to higher antibody titers, and a median of 15 days from symptom

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179   resolution to higher antibody titers. This suggests that the optimal time frame for widespread

180   antibody testing is at least three to four weeks after symptom onset and at least two weeks after

181   symptom resolution. In our survey, we did not find evidence for a decrease in IgG antibody titer

182   levels on repeat sampling.

183   Although we do not yet know what, if any, immunity is conferred by IgG or the duration of the

184   IgG response, at this time it seems likely that IgG to SARS-CoV-2 may confer some level of

185   immunity based on what is known about viral immunity to other pathogens. In prior studies of

186   SARS-CoV-1 and Middle East Respiratory Syndrome patients, IgG peaked within months of

187   primary infection and waned over time.[11-13] Similar observations have been made with human

188   coronaviruses were immunity can confer at least limited protection.[13] In order to study the

189   duration of IgG antibody response to SARS-CoV-2, we plan to follow our cohort for the next six

190   months in order to track titer levels.

191   Among participants who did not have prior PCR but who were deemed high risk, i.e., people

192   with symptoms consistent with SARS-CoV-2 who were told by a healthcare provider they had

193   presumed infection, lived with someone with confirmed infection, or were healthcare workers

194   themselves, we found 36% of this population had IgG antibodies to SARS-CoV-2. This finding

195   suggests that a majority of participants suspected of having Covid-19 actually were not infected

196   with SARS-CoV-2; however, it may also include a false negative rate of our assay (which has a

197   92% sensitivity) or insufficient time for participants to mount an IgG antibody response. This

198   underscores the importance of expanded PCR testing in order to improve diagnosis of this

199   disease even in minimally symptomatic individuals.

200   The 19% of participants who remained PCR positive despite self-reporting full resolution of

201   symptoms bring to light important considerations regarding the possible duration of viral

202   transmission, and the limited utility of PCR testing to ensure clearance. This positive PCR

203   finding could represent shedding of nonviable virus, non-infectious genome fragments or viruses

204   engulfed by immune cells, asymptomatic carriers of SARS-CoV-2, or ongoing disease despite

205   full resolution of symptoms. Detection of viral genome even months after resolution of infection

206   has been shown for viruses like measles virus.[14] Further studies are warranted to determine if

207   nasopharyngeal PCR positivity is related to transmission, and if so, for how long. This will have

208   significant implications in terms of guidance of when recovered SARS-CoV-2 individuals should

209   end self-isolation, currently recommended at least seven days after symptom onset with at least

210   72 hours without fever off of antipyretics. If PCR positivity is a result of identifying

211   noninfectious genome or nonviable virus, it may be necessary to avoid use of PCR as a definition

212   of clearance in SARS-CoV-2.

213   There are limitations to our evaluation. All participants had mild disease, and thus these data

214   may not reflect PCR or Ab findings in a moderately or severely ill population. Participants were

215   recruited based on self-referral and self-reporting, which may have led to recall bias in terms of

216   dates of symptom onset, resolution and duration, and may have led us to miss asymptomatic

217   carriers who did not inquire about testing. Additionally, given recruitment via an English-

218   language online survey and our use of a single collection site, our sample likely included more

219   recovered participants of younger ages with internet access and the ability to travel to our site for

220   testing. Furthermore, we did not collect rigorous data regarding symptom severity which could

221   potentially be related to the timeline and strength of IgG antibody response to SARS-CoV-2.

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222   Future studies are planned to help us understand the magnitude and duration of the IgG response

223   in patients recovered from SARS-CoV-2, and what antibody titer may be necessary to protected

224   individuals from reinfection. We also hope to better understand which, if any, patients do not

225   mount an IgG immune response. Finally, the clinical significance of prolonged positive SARS-

226   CoV-2 nasopharyngeal PCR in the absence of symptoms requires further clarification.

227   **Conclusion**

228   Duration of nasopharyngeal PCR SARS-CoV-2 PCR detection and time to mount IgG antibody

229   response have important implications for the spread of this virus and risk for re-infection among

230   individuals. In our sample, we found that 19% of people continue to have nasopharyngeal PCR

231   positivity two or more weeks after symptom resolution, and that it takes three or more weeks to

232   mount an IgG antibody response believed to be potentially protective against future infection.

233   Reassuringly, we found that almost all participants with confirmed SARS-CoV-2 infection in our

234   study mounted an IgG immune response to this disease. Taken together, these findings will be

235   pivotal in understanding disease activity of SARS-CoV-2 moving forward.

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Table 1.

	Respondents with past PCR+ confirmed SARS-CoV-2				
	All (n=624)	Ab+ (511)	Ab Weak + (42)	Ab- (71)	p-value
Age, Mean (SD)	39.14 (12.09)	39.11 (11.94)	38.10 (12.28)	40.00 (13.19)	0.716
Age, Range	17 - 76	17 - 76	18 - 67	19 - 74	
Age by Category, N (%)					0.117
17-29	144 (23%)	110 (22%)	15 (36%)	19 (27%)	
30-59	432 (69%)	364 (71%)	24 (57%)	44 (62%)	
60+	48 (8%)	37 (7%)	3 (7%)	8 (11%)	
Gender					0.002
Male, N (valid %)	368 (59%)	303 (60%)	33 (81%)	32 (46%)	
Female, N (valid %)	252 (41%)	206 (40%)	8 (19%)	38 (54%)	
Test Results, N (%)					
All Still PCR+ at next test	217 (35%)	159 (31%)	22 (52%)	36 (51%)	
PCR+/Ab+	159 (26%)	-	-	-	-
PCR-/Ab-	18 (3%)	-	-	-	-
PCR-/Ab+	95 (15%)	-	-	-	-
PCR+/Ab-	36 (6%)	-	-	-	-
PCR-/Abweak+	7 (1%)	-	-	-	-
PCR+/Abweak+	22 (4%)	-	-	-	-
No new PCR/Ab+	257 (41%)	-	-	-	-
No new PCR/Abweak+	13 (2%)	-	-	-	-
No new PCR/Ab-	17 (3%)	-	-	-	-
Time from last PCR+ to Current Result, Median days (Range)	19 (8-54)	19 (8-49)	18 (8-26)	17 (10-54)	0.004
Symptoms, Median days (Range)					
Onset to Result	23 (3-46)	23 (1-46)	20 (15-28)	21 (14-34)	<0.001
Resolution to Result	14 (4-49)	14 (-1-49)	13.5 (4-26)	12 (4-25)	0.013
Symptom Duration	9 (1-31)	9 (1-31)	7 (2-14)	10 (2-27)	0.017
	Respondents with NO past PCR+, suspected SARS-CoV-2				
	All (n=719)	Ab+ (250)	Ab Weak + (19)	Ab- (450)	p-value
Age, Mean (SD)	41.39 (12.16)	39.49 (12.76)	41.53 (12.73)	42.44 (11.69)	0.009
Age, Range	17 - 76	17 - 76	27 - 71	21 - 75	
Age by Category, N (%)					0.008
17-29	112 (16%)	55 (22%)	4 (21%)	53 (12%)	
30-59	536 (75%)	174 (70%)	12 (63%)	350 (78%)	
60+	71 (10%)	21 (8%)	3 (16%)	47 (10%)	
Gender					0.388
Male, N (valid %)	338 (47%)	123 (49%)	11 (58%)	204 (45%)	

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Female, N (valid %)	381 (53%)	127 (51%)	8 (42%)	246 (55%)	
Live with someone tested pos?	62 (9%)	33 (13%)	1 (5%)	28 (6%)	0.061
Had direct patient contact?	69 (10%)	24 (10%)	1 (5%)	44 (10%)	0.293
Told by doctor had COVID-19?	264 (23%)	115 (46%)	10 (53%)	139 (31%)	0.020
Test Results, N (%)					
PCR+/Ab+	24 (3%)	-	-	-	
PCR-/Ab-	174 (24%)	-	-	-	
PCR-/Ab+	38 (5%)	-	-	-	
PCR+/Ab-	7 (1%)	-	-	-	
PCR-/Abweak+	3 (1%)	-	-	-	
PCR+/Abweak+	1 (0%)	-	-	-	
No PCR/Ab+	188 (26%)	-	-	-	
No PCR/Abweak+	15 (2%)	-	-	-	
No PCR/Ab-	269 (37%)	-	-	-	
Symptoms, Median days (Range)					
Onset to Result	28 (3-70)	26 (7-50)	26 (22-30)	32.5 (3-70)	<0.001
Resolution to Result	19 (5-70)	18 (5-34)	17 (10-57)	21.5 (5-70)	<0.001
Symptom Duration	9 (2-67)	8 (2-67)	10 (5-21)	10 (2-43)	0.021

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248 **Table 2.**

	Antibody Pts with 2nd Ab test as of 4/24 (N=64)		
	Test 1	Test 2	Test 3
	*1st test restricted to 4/10 or earlier	*2nd test 4/22 or earlier	
Antibody result, N (%)			
positive	-	48 (75%)	2 (3%)
weakly positive	19 (30%)	13 (20%)	-
negative	45 (70%)	3 (5%)	-
Time Bw results 1 & 2, Median (Range)	13 (5-25)		-
Change in result, N (%)			
neg to pos	33 (52%)	-	-
neg to weak pos	9 (14%)	-	-
neg to neg	3 (5%)	-	-
weak pos to pos	15 (23%)	-	-
weak pos to neg	0 (0%)	-	-
weak pos to weak pos	4 (6%)	-	-

Time bw results by result change, Median (Range)			
neg to pos	14 (5-22)	-	-
neg to weak pos	12 (6-19)	-	-
neg to neg	14 (12-15)	-	-
weak pos to pos	11 (7-16)	-	-
weak pos to neg	-	-	-
weak pos to weak pos	14.5 (13-18)	-	-
Symptoms, Median days (Range)			
Onset to Result	19 (2-31)	31.5 (20-42)	-
Resolution to Result	12 (4-25)	24 (12-32)	-
Symptom Duration	5 (2-27)	-	-

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252   **Table 3.**
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	PCR Pts with 2nd PCR test as of 4/24 (N=182)		
	Test 1	**Test 2**	**Test 3**
PCR result, N (%)	*1st test restricted to 4/10 or earlier	*2nd test 4/22 or earlier	
Detected	182 (100%)	70 (39%)	8 (57%)
Not Detected	-	112 (61%)	6 (43%)
Time Bw results 1 and 2, Median (Range)	10 (3-21)	-	-
Change in result, N (%)			
Detected to Detected	70 (39%)	-	-
Detected to Not Detected	12 (61%)	-	-
Time bw results by result change, Median (Range)			
Detected to Detected	10 (3-21)	-	-
Detected to Not Detected	10 (4-21)	-	-
Symptoms, Median days (Range)			
Onset to Result	20 (1-43)	30 (6-50)	-
Resolution to Result	12 (-1-20)	22 (4-34)	-
Symptom Duration	8 (1-27)	-	-

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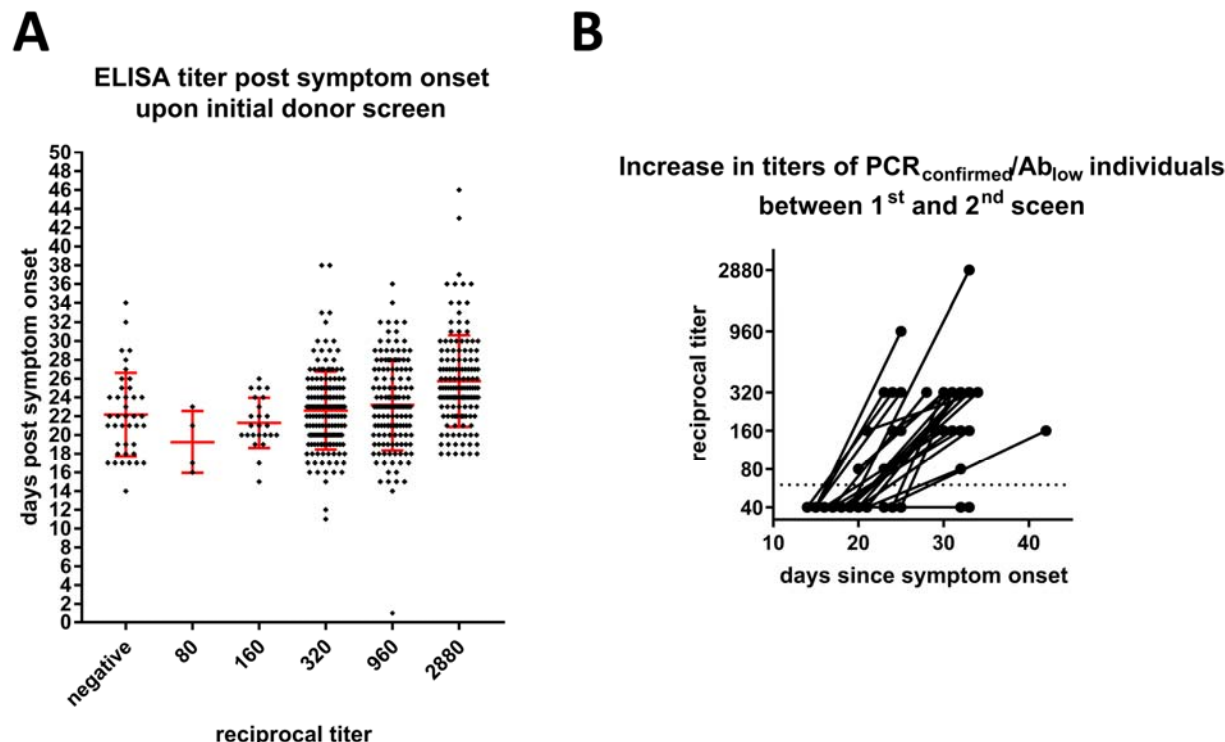265
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267 **Figure Legends**

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269 **Figure 1: Antibody responses in PCR-confirmed COVID19 individuals. A** shows antibody
270 testing results days post symptom onset stratified into titer categories. The highest titer category
271 contains both 1:2880 and >1:2880 titers. Bars represent the mean; error bars the standard
272 deviation. **B** Individuals with negative titers were recalled for retesting. Both the original test
273 result and the second test result post day onset are shown. Negative titers were assigned a value
274 of 1:40 for representation purpose, the line represents the cut-off between positive and negative
275 titers. Only results for individuals for which date of symptom onset is known are shown.
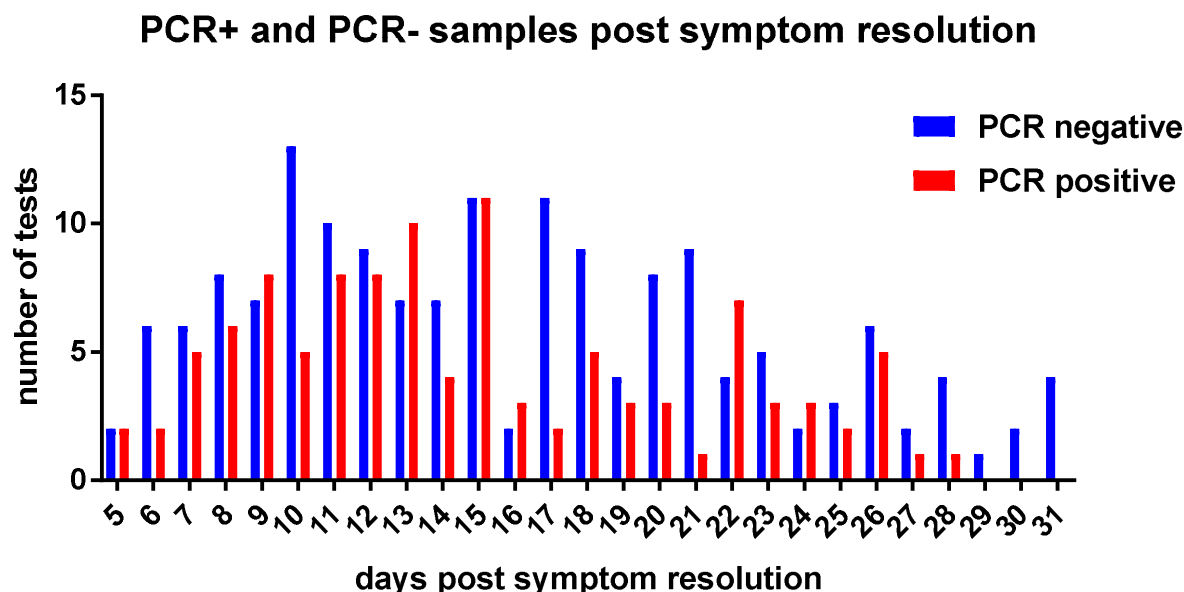
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Figure 2: PCR results of initially PCR-confirmed COVID19 individuals. Viral genome was
detected in nasopharyngeal swaps of individuals screened as plasma donors. Number of subjects
positive or negative days post symptom resolution are shown. More than one result from an
individual might be shown if tested more than once on different days. Only results for subject for
which a date of symptom resolution was available are shown.

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326