

1 **SARS-CoV-2 RNA shedding in recovered COVID-19 cases and the presence of antibodies**  
2 **against SARS-CoV-2 in recovered COVID-19 cases and close contacts**

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34

## 35 **Abstract**

36           Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome  
37 coronavirus 2 (SARS-CoV-2). COVID-19 emerged in December 2019 and has spread globally.  
38 Although Thailand has been effective at controlling the spread of COVID-19, disease  
39 surveillance and information on antibody responses in infected cases and close contacts are  
40 needed because there is still no specific treatment or vaccine available. We investigated 217  
41 recovered COVID-19 cases to monitor their viral RNA shedding and production of antibodies  
42 against SARS-CoV-2. The presence of antibodies in blood samples from 308 close contacts of  
43 COVID-19 cases was also determined. Viral RNA was still detectable in 6.6 % of recovered  
44 COVID-19 cases. The most prolonged duration of viral RNA shedding detected in this study was  
45 105 days. IgM, IgG, and IgA antibodies against SARS-CoV-2 were detected in 13.82, 88.48, and  
46 83.41 % of the recovered cases 4–12 weeks after disease onset, respectively. Although the  
47 patients had recovered from their illness, the levels of antibodies detected showed association  
48 with their symptoms during their stay in hospital. Fifteen of the 308 contacts (4.87 %) of  
49 COVID-19 cases tested positive for IgG antibodies. The presence of antibodies against SARS-  
50 CoV-2 suggested that there was viral exposure among close contacts. Viral clearance and the  
51 pattern of antibody responses in infected individuals are both crucial for effectively combatting  
52 SARS-CoV-2. Our study provides additional information on the natural history of this newly  
53 emerging disease related to both natural host defenses and a strategy for vaccine development.

54

## 55 **Introduction**

56           Coronavirus disease 2019 (COVID-19) is an emerging infectious disease caused by a  
57 novel coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The  
58 disease emerged in December 2019 and has since spread globally. On March 11, 2020, the  
59 World Health Organization (WHO) declared the coronavirus outbreak to be a pandemic. Currently,  
60 over 11 million cases of COVID-19 have been reported worldwide, resulting in more than  
61 500,000 deaths [1]. The number of confirmed COVID-19 cases reported in Thailand was 3,195,  
62 with 58 deaths [2]. In Thailand, the first case of COVID-19 was detected in the capital, Bangkok,  
63 in mid-January 2020. During the first few months, most of the reported cases in Thailand were  
64 associated with travelers who had visited other countries. However, the number of reported cases  
65 increased rapidly owing to the spreading of the disease *via* entertainment venues and Thai boxing  
66 stadiums.

67           SARS-CoV-2 is an enveloped, positive-sense single-stranded RNA virus of the  
68 Coronaviridae family. It was classified as a novel betacoronavirus similar to the previously  
69 identified SARS-CoV (severe acute respiratory syndrome coronavirus) and MERS-CoV (Middle  
70 East severe respiratory syndrome coronavirus) [3,4]. SARS-CoV-2 enters host cells through the  
71 binding of its spike glycoprotein to the host angiotensin-converting enzyme 2 (ACE2) receptor.  
72 This virus can spread across species. The reproductive number ( $R_0$ ) of SARS-CoV-2 is higher  
73 than that of SARS-CoV and MERS-CoV, suggesting that this novel coronavirus has a greater  
74 pandemic potential [5–7]. Although the number of reported COVID-19 cases in Thailand has  
75 decreased rapidly [2], the surveillance of infected cases and disease transmission is crucial for  
76 disease prevention before a vaccine can be developed.

77 SARS-CoV-2-infected patients present with a wide range of symptoms, ranging from  
78 mild to severe. Severe illness includes pneumonia, difficulty breathing, and respiratory failure.  
79 SARS-CoV-2-infected individuals can also be asymptomatic [8].

80 Real-time reverse transcription-polymerase chain reaction (RT-PCR) for viral RNA  
81 detection is widely used for the laboratory-based diagnosis of COVID-19. Antibody detection  
82 has also been increasingly reported to indicate exposure to SARS-CoV-2. Although several  
83 reports have suggested the combined use of antibody and viral RNA detection, real-time RT-  
84 PCR is currently the method-of-choice for SARS-CoV-2 diagnosis [9–11].

85 Viral RNA has been shown to be detectable in recovered patients, although the duration  
86 of viral RNA shedding reported varied among infected patients. Viral RNA shedding can last at  
87 least 6 weeks [12–16]. Liu et al. reported that patients with severe symptoms had a higher viral  
88 load and a longer viral shedding period than patients with mild symptoms (<10 days for the  
89 group with mild symptoms and >10 days for the group with severe symptoms). These authors  
90 investigated viral RNA in nasopharyngeal (NP) swabs and the delta Ct values were used to  
91 represent the viral load [17].

92 Antibody responses following infection can be utilized for disease diagnosis and can also  
93 represent evidence of viral exposure. Detection of IgM, IgG, and IgA antibodies against SARS-  
94 CoV-2 antigens in COVID-19 have been reported. Although antibody detection is currently not  
95 widely used for COVID-19 diagnosis, the presence of antibodies provides additional valuable  
96 information. The presence of antibodies is indicative of immune responses to SARS-CoV-2,  
97 immune clearance, and pathogenesis in infected patients. Additionally, the presence of antibodies  
98 reveals the transmission of the virus to contacts.

99           In this study, we followed recovered COVID-19 cases using real-time RT-PCR to  
100 investigate the duration of viral shedding as detected in NP swabs. Antibody detection was  
101 performed to monitor antibody levels in all the recovered patients. Blood samples from  
102 individuals who had a history of contact with COVID-19 patients were also collected for  
103 antibody detection.

104

## 105 **Materials and methods**

106 The study protocol was approved by the Institutional Ethics Committee (IRB) of the  
107 Bangkok Metropolitan Administration, Thailand (IRB No. M001h/63\_Exp). The IRB waived the  
108 need for consent because the used samples were obtained from routine preventive measures and  
109 were de-identified and anonymous.

110

## 111 **Samples and sample collection**

112 Samples included in this study were collected from recovered COVID-19 patients and  
113 their close contacts. The recovered COVID-19 cases recruited in this study were patients who  
114 had been diagnosed with SARS-CoV-2 infection and admitted to hospitals or public health  
115 centers under the Bangkok Metropolitan Administration. NP swabs and blood samples were  
116 collected for the detection of viral RNA and determination of the presence of antibodies,  
117 respectively. Close contacts were individuals with a history of contact with COVID-19 patients.  
118 Only blood samples for antibody detection were collected from close contacts.

119 For recovered cases, the duration between the first day of symptom onset to the day of  
120 sample collection was recorded. For close contacts, the duration between the last contact and  
121 sample collection was noted.

122

## 123 **Real-time RT-PCR for the detection of viral RNA**

124 Real-time RT-PCR targeting the RNA-dependent RNA polymerase (RdRp), envelope  
125 (E), and nucleocapsid (N) genes of SARS-CoV-2 was performed using the LightMix Modular  
126 SARS and Wuhan CoV E-gene Kit (TIB MOBIOL, Syntheselabor GmbH, Berlin, Germany) in a

127 LightCycler 480 II system following the manufacturer's recommendation (Roche Diagnostics  
128 International Ltd., Rotkreuz, Switzerland). The results were reported as detectable or  
129 undetectable based on Ct values.

130

## 131 **Detection of antibodies against SARS-CoV-2 by enzyme-linked**

### 132 **immunoassays**

133 For recovered COVID-19 cases, IgM, IgG, and IgA antibodies against SARS-CoV-2  
134 were determined, whereas, for close contacts, IgG antibodies were investigated in blood samples.  
135 IgM antibody levels were measured using the MAGLUMI 2000 fully automatic  
136 chemiluminescent analytical system (Snibe, Shenzhen, China) according to the manufacturer's  
137 instructions. The values were reported as arbitrary unit per milliliter (AU/mL). The  
138 determination of IgG and IgA antibodies was performed using an ELISA automated system  
139 (Euroimmun, Luebeck, Germany) following the manufacturer's instructions. The optical density  
140 (OD) at 450 nm was measured and the ratio was calculated using the reading of each sample to  
141 the reading of the calibrator. The result was obtained as a ratio between sample and cut-off OD  
142 value. Sample to cut off value of  $> 1$  was considered seropositive.

143

### 144 **Statistical analysis**

145 The association between antibody levels and clinical characteristics was analyzed using  
146 the Mann–Whitney *U*-test and chi-square test. The difference between the number of people  
147 positive for IgG antibodies against SARS-CoV-2 in each group of close contacts was determined  
148 using the chi-square test. A *p*-value  $< 0.05$  was considered statistically significant.



## 149 **Results**

150 A total of 217 recovered COVID-19 cases were recruited in this study. Based on the  
151 patients' records during their stay in hospital, the clinical manifestations of the recovered  
152 COVID-19 cases were considered to be asymptomatic (4/217), mild (151/217), presenting with  
153 pneumonia (59/217), and presenting with pneumonia with tracheal intubation (3/217). A total of  
154 308 close contacts were included in this study. Of these, 118 were household contacts, while the  
155 rest were close friends, colleagues, health care personnel who took care of the COVID-19 cases,  
156 taxi drivers, neighbors, or individuals who lived or performed activities in the same community  
157 as the COVID-19 cases.

158 All samples were collected from April to June 2020. Gender, age, and other information  
159 relating to the recovered COVID-19 cases and close contacts gathered in this study are shown in  
160 Table 1.

161

162 **Table 1. Recovered COVID-19 cases and close contacts included in this study and the**  
163 **results of antibodies detection.**

	<b>Recovered cases</b>	<b>Close contacts</b>
<b>Total number</b>	217	308
<b>Age (years)</b>	2–76	2–77
<b>Gender</b>		
Number of males (age in years)	92 (2–76)	159 (2–69)
Number of females (age in years)	125 (11–70)	149 (7–77)
<b>History of symptoms</b>		

Mild/asymptomatic; number (%)	155/217 (71.43)	NA
Pneumonia; number (%)	59/217 (27.19)	
Pneumonia with intubation; number (%)	3/217 (1.38)	
<b>Duration from symptom onset to sample collection (days)</b>	28–142	NA
<b>Duration from the last contact to sample collection (days)</b>	NA	1–128
<b>IgM antibody detection</b>		
Number of positive cases (%)	30/217 (13.82)	ND
Duration from symptom onset to sample collection for positive cases (days)	34–67	
<b>IgG antibody detection</b>		
Number of positive cases (%)	192/217 (88.48)	15/308 (4.87)
Duration from symptom onset to sample collection for positive case (days)	28–142	
<b>IgA antibody detection</b>		
Number of positive cases (%)	181/217 (83.41)	ND
Duration from symptom onset to sample collection for positive cases (days)	28–142	

164 NA: not applicable; ND: not done.

165

166 **Detection of SARS-CoV-2 RNA in recovered COVID-19 cases**

167 NP swabs could not be obtained for 5 of the 217 recovered COVID-19 cases recruited in  
168 this study. Among the 212 cases whose NP swabs were collected, 14 (6.6 %) showed detectable  
169 viral RNA by real-time RT-PCR. The ages of these 14 cases ranged from 16–67 years. The Ct  
170 values obtained from the real-time RT-PCR analysis indicated the low amount of viral RNA left.  
171 RT-PCR analysis of NP swabs from 2 of the 14 recovered cases showed Ct values of 28.43 and  
172 29.61. The Ct values obtained from the NP swabs of the other 12 cases ranged between 30.22  
173 and 37.74. The duration between the day of first onset and sample collection ranged from 36–  
174 105 days (mean = 57 days). Our study suggested that viral RNA shedding was detectable for up  
175 to 15 weeks.

176

## 177 **Antibodies against SARS-CoV-2 in recovered COVID-19 cases**

178 The levels of IgM, IgG, and IgA antibodies against SARS-CoV-2 in blood samples from  
179 217 recovered cases were measured. Fig 1A–C depicts the analysis of the data from the  
180 determination of the three antibody isotypes against SARS-CoV-2 according to the duration (in  
181 weeks) of the first day of symptom onset to the day of sample collection. Among the 217 cases,  
182 30 (13.82 %), 192 (88.48 %), and 181 (83.41 %) were positive for IgM, IgG, and IgA antibodies,  
183 respectively. All 30 IgM-positive samples were also positive for IgG and IgA antibodies. In  
184 addition, 150/217 (69.12 %) cases were positive for both IgG and IgA antibodies. The duration  
185 between the day of the first symptom onset and blood sample collection varied from 28–142  
186 days. As shown in Table 1, although only 13.82 % of recovered cases were IgM-positive, IgM  
187 antibodies remained detectable for up to 2 months in some cases. IgG and IgA antibodies were  
188 detectable for up to 20 weeks.

189

190 **Fig 1. Antibodies against SARS-CoV-2 in recovered COVID-19 cases.** IgM (A), IgG (B), and  
191 IgA (C) antibodies against SARS-CoV-2 in recovered COVID-19 cases were determined as  
192 mentioned in the Materials and methods. The data represent the results according to the duration  
193 (weeks) between the first day of symptom onset and the day of sample collection. Bars represent  
194 median values (middle line) and the upper and lower interquartile range (IQR) (upper and lower  
195 lines).

196

197 As mentioned above, COVID-19 symptoms included asymptomatic, mild, pneumonia,  
198 and pneumonia with tracheal intubation. To investigate the association of patient symptoms with  
199 the findings of this study, patient symptoms were grouped into nonpneumonia (asymptomatic  
200 and mild symptoms; 155/217 cases, mean age = 34.5) and pneumonia (62/217 cases, mean age =  
201 42.2) groups. The ages of cases were significantly different between the groups with and without  
202 pneumonia (chi-square,  $p = 0.027$ ). Moreover, when the association between antibody  
203 concentrations and symptoms was analyzed, the levels of IgM, IgG, and IgA antibodies against  
204 SARS-CoV-2 were significantly higher in the pneumonia group than in the nonpneumonia group  
205 ( $p = 0.001$ ,  $p < 0.0001$ , and  $p < 0.0001$ , respectively) (Fig 2A–C).

206

207 **Fig 2. The association between the antibodies against SARS-CoV-2 and symptoms of**  
208 **recovered COVID-19 cases.** The levels of IgM (A), IgG (B), and IgA (C) antibodies against  
209 SARS-CoV-2 in recovered COVID-19 cases with and without pneumonia are shown. Bars  
210 represent median values (middle line) and  $1\times$  the upper and lower interquartile range (IQR)  
211 (upper and lower lines). The levels of IgM, IgG, and IgA antibodies against SARS-CoV-2 were

212 higher in patients with pneumonia than in those without pneumonia. \*  $p = 0.0002$ , \*\*  $p <$   
213  $0.00001$ .

214

### 215 **Antibody detection in close contacts of SARS-CoV-2 patients**

216 IgG antibodies against SARS-CoV-2 detected in close contacts of COVID-19 patients are  
217 shown in Fig 3A. Among the 308 close contacts, 15 (4.87 %) were positive for IgG antibodies,  
218 and two of these 15 close contacts presented with respiratory symptoms. Blood samples from  
219 healthy donors collected in 2018, i.e., before the emergence of COVID-19, were tested for the  
220 presence of antibodies against SARS-CoV-2. All 50 samples showed negative results.

221

#### 222 **Fig 3. IgG antibodies against SARS-CoV-2 in close contacts of COVID-19 cases.** IgG

223 antibodies against SARS-CoV-2 in blood samples from 308 close contacts of COVID-19 cases  
224 and 50 healthy controls collected in 2018 were determined. IgG antibodies were detected in  
225 15/308 close contacts, but none of the healthy controls (A). The numbers of IgG-positive  
226 household and nonhousehold contacts are shown (B).

227

228 Of the 15 IgG-positive contacts, 11 (73.33 %) were household contacts and 4 (26.67 %)  
229 were colleagues and/or friends of the COVID-19 cases (Fig 3B). Our data supported that  
230 household contacts represent a high-risk group for disease exposure. A total of 118 household  
231 contacts were recruited in this study. Our results showed that the prevalence of viral exposure  
232 among the household contacts, as evidenced by the presence of antibodies, was 9.3 % (11 out of  
233 118 contacts). The prevalence of viral exposure among the other types of close contacts was 2.1  
234 % or 4 out of 190 contacts (chi-square,  $p = 0.015$ ).

## 235 **Discussion**

236           The number of cases of COVID-19 has increased rapidly worldwide since the emergence  
237 of SARS-CoV-2 infection in December 2019. Laboratory diagnosis and treatments have been  
238 urgently evaluated. In addition, the duration of viral RNA shedding and patterns of immune  
239 responses, especially antibody production, have been studied. The report of a COVID-19 case in  
240 Taiwan showed that SARS-CoV-2 RNA could be detected on day 5 after disease onset. IgM  
241 antibodies have also been detected on day 11, and are still detectable on day 27 [18]. Xiang et al.  
242 demonstrated the dynamics of antibody responses in samples collected from COVID-19 cases at  
243 3–40 days after symptom onset. The authors reported that IgM and IgG antibodies against  
244 SARS-CoV-2 could be detected as early as the fourth day after symptom onset. In confirmed  
245 cases, the sensitivity of the IgM and IgG tests were 77.3 and 83.3 %, respectively [19]. Nisreen  
246 et al. assessed the levels of IgG and IgA antibodies in one severe and two mild cases of COVID-  
247 19, and found that the concentrations of these antibodies were higher in severe cases. Samples  
248 were collected between 6 and 27 days after diagnosis, and the samples from the severe case  
249 showed earlier and higher seroconversion [20]. The same finding was demonstrated in MERS-  
250 CoV infection [21,22]. A study on SARS-associated coronavirus showed that IgG antibodies  
251 persist longer than IgA antibodies and are therefore more suitable for disease surveillance [23].  
252 A different report from Finland showed that the neutralizing antibodies, IgM and IgG, could be  
253 detected within 9 days of symptom onset. The antibodies were still detectable on day 20 after  
254 symptom onset [24].

255           Long et. al. reported that IgG and IgM antibody concentrations were higher in severe  
256 cases of COVID-19, while IgM levels decreased slightly after the third week of symptom onset.  
257 This study followed up 63 patients by collecting serum at 3-day intervals, and 96.8 % of the

258 patients showed seroconversion. In addition, 26 patients who were initially negative for IgG and  
259 IgM antibodies were followed up for the detection of seroconversion. The median number of  
260 days for IgG and IgM seroconversion was 13 days after symptom onset. Both IgG and IgM  
261 antibodies were detected in the in 9/26 samples, IgM was detected earlier than IgG in 7/26  
262 patients, and later in 10/26 cases [25]. Yongchen et al. reported the longitudinal follow-up by  
263 viral RNA and antibody testing of 5 severe, 11 non-severe, and 3 asymptomatic COVID-19  
264 cases. Viral RNA remained detectable for 9–33 days (median = 14), 2–21 days (median = 10),  
265 and 5–28 days (median = 5) in severe, non-severe, and asymptomatic cases, respectively. All  
266 symptomatic patients in this study showed antibody responses. Three patients showed  
267 seroconversion within the first week. Moreover, this study showed that the antibody to SARS-  
268 CoV-2 was detectable for at least 6 weeks. However, only 20 % (1/5) of asymptomatic cases  
269 showed an antibody response, with this one patient showing seroconversion in the third week  
270 after diagnosis [26]. Zhao et al. reported the dynamics of total, IgM, and IgG antibodies against  
271 SARS-CoV-2 with disease progression in 173 COVID-19 cases. Less than 40 % of the patients  
272 showed detectable antibodies within the first week after symptom onset. On day 15 after disease  
273 onset, 100 %, 94.3 %, and 79.8 % of cases were positive for total, IgM, and IgG antibodies,  
274 respectively. IgM appeared before IgG (median time to seroconversion was 12 and 14 days,  
275 respectively). This study showed that the sensitivity of IgM antibody detection was higher than  
276 for IgG antibodies. Viral RNA detection showed the greatest sensitivity during the first week of  
277 symptom onset. In addition, this study showed that high antibody titers were associated with the  
278 worst clinical manifestations [27].

279 Close contacts of COVID-19 cases have been previously investigated. Bi et al.  
280 investigated 391 COVID-19 cases and 1,286 close contacts, and showed that household contacts

281 and individuals who traveled with COVID-19 cases had a high risk of infection [28]. Meanwhile,  
282 Guo et al. showed that the median duration of antibody detection was 5 days for IgM and IgG  
283 and 14 days for IgG. In this study, samples were collected between 1–39 days after disease onset.  
284 These authors also investigated four close contacts of a family with 2 COVID-19 cases, and  
285 found that these 4 close contacts were asymptomatic. IgM antibodies were detected in 3 of the 4  
286 close contacts, whereas IgG antibodies were undetectable [29].

287 In the current study, we demonstrated that viral shedding could still be detected in 14/212  
288 (6.6 %) of the recovered COVID-19 cases. The longest duration of viral shedding shown in our  
289 study was 105 days. Several reports have indicated that the antibody levels persisted for at least 6  
290 weeks. We chose to follow the recovered cases to investigate the persistence of antibodies  
291 against SARS-CoV-2. The IgM level, which commonly declines rapidly following infection, was  
292 still detectable in 13.82 % of recovered cases. IgA, an immune component of mucosal immunity,  
293 and IgG were still detectable in more than 80 % of recovered cases. It would be interesting to  
294 investigate further the recovered cases whose antibodies against SARS-CoV-2 were  
295 undetectable.

296 We also found that high levels of antibodies were associated with disease severity.  
297 Interestingly, although we investigated the antibody levels in the recovered cases, an association  
298 between high antibody levels and severe cases was still observed. This suggests that patients  
299 with high antibody levels could have a higher viral burden. However, there is still no clear  
300 evidence to indicate that a correlation exists between the number of antibodies produced and  
301 viral load. A high number of antibodies should facilitate viral clearance and alleviate disease  
302 symptoms. However, the roles of the antibodies produced in COVID-19 cases remain to be  
303 elucidated.



304 In this study, we investigated a large group of close contacts, and found that 4.8 % of  
305 them displayed evidence of viral exposure. Besides, our results showed that household contact  
306 represent a high-risk group for disease exposure which is essential for the prevention of disease  
307 transmission.

308 In conclusion, our study provided information on COVID-19 cases after disease  
309 recovery. The data suggested that further monitoring should be performed to achieve a full  
310 understanding of the duration of viral clearance and antibody responses. Our study, which  
311 investigated both recovered cases and close contacts, was intended to support the policy followed  
312 by Thailand on the prevention of the spread of SARS-CoV-2 infection in the country. Our  
313 findings regarding the high antibody response in recovered patients will be useful for the  
314 recruitment of volunteers by the National Blood Center for the donation of convalescent plasma.  
315 Additionally, our data are useful for the further understanding of COVID-19 transmission and  
316 infection control, while our results on the patterns of antibody production and duration of  
317 antibody detection may inform strategies for achieving herd immunity and as well as for vaccine  
318 immunization.

319

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324 interesting information obtained from this study could be gathered for future development of  
325 Covid-19 therapeutic and vaccine strategies.

326

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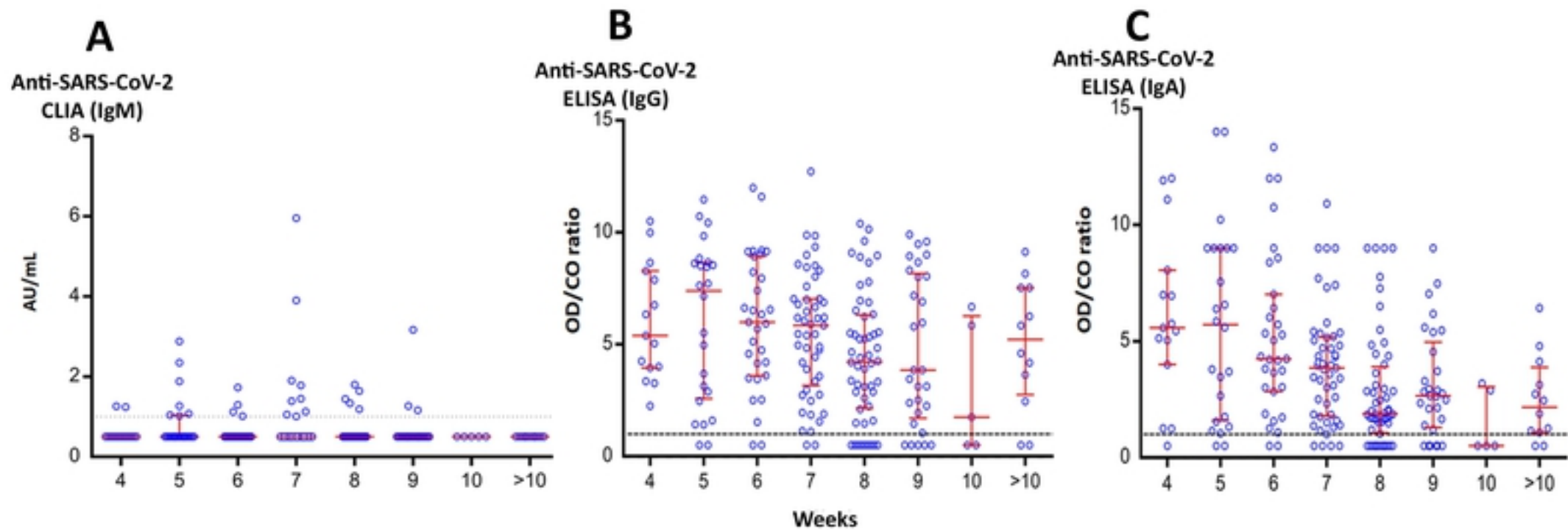


Figure 1

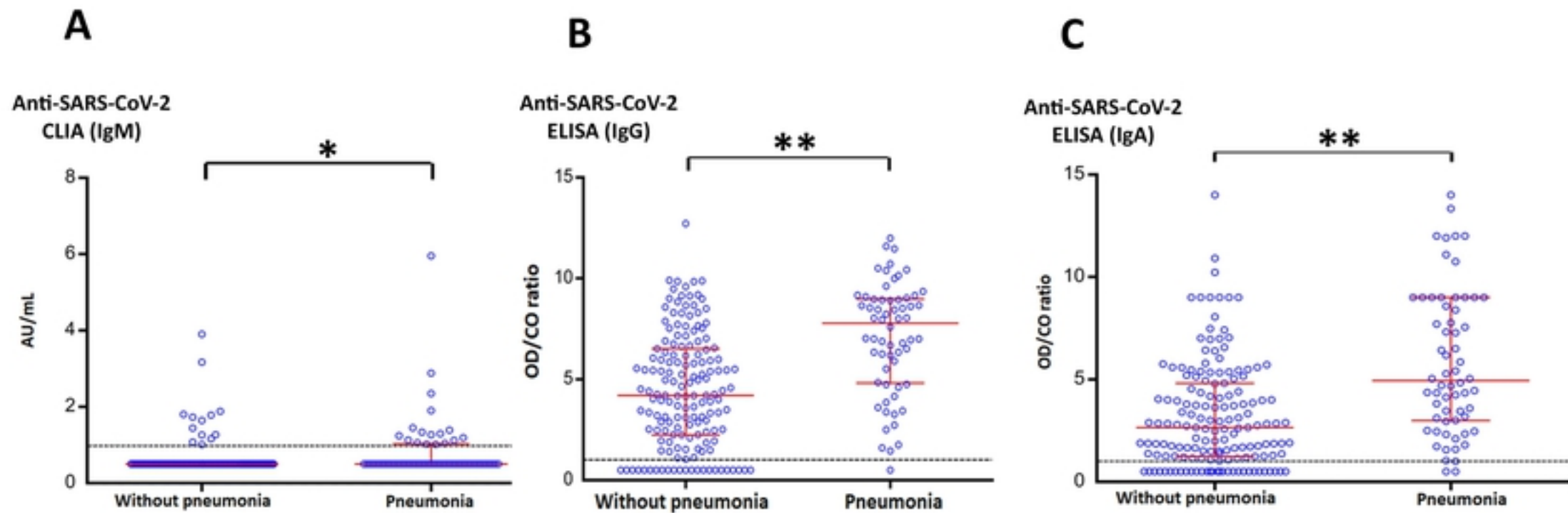
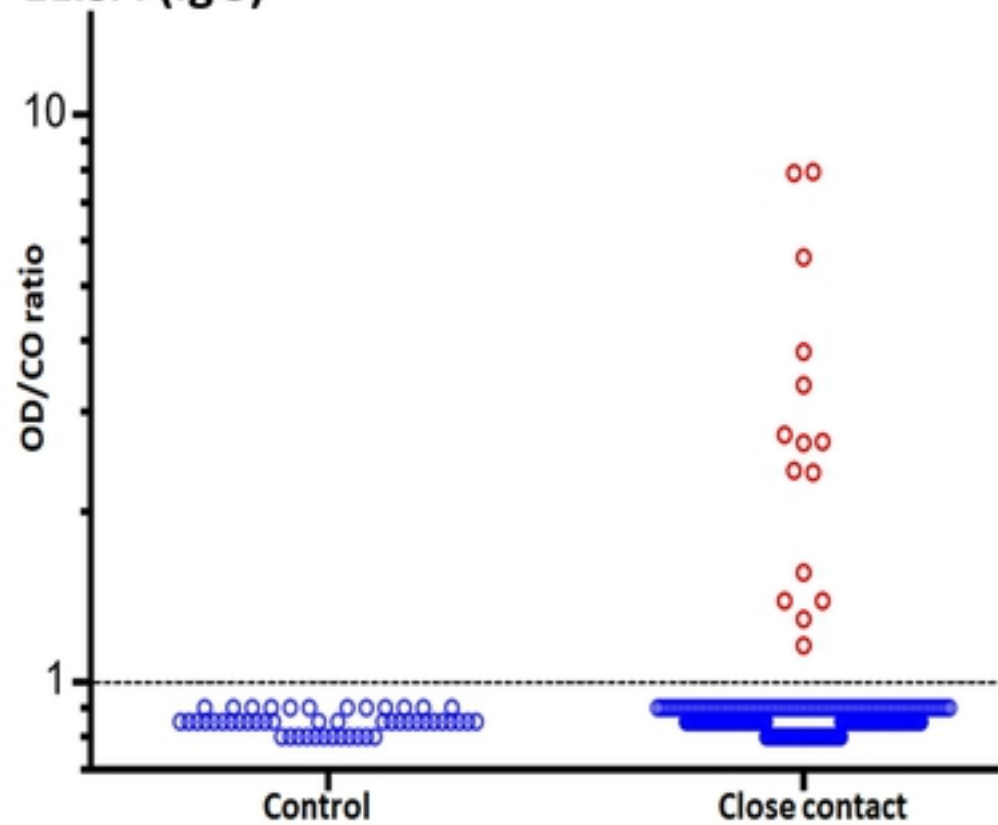


Figure 2



**A**  
Anti-SARS-CoV-2  
ELISA (IgG)



**B**  
Number

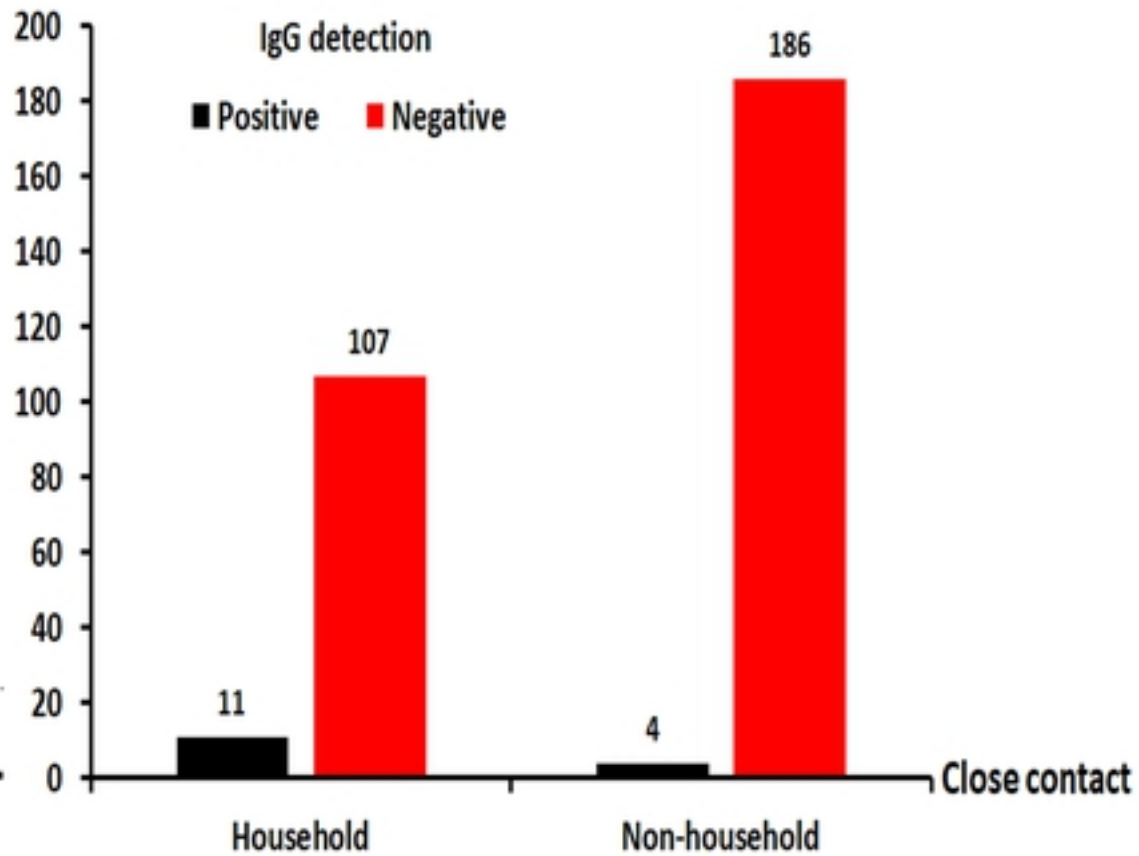


Figure 3