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TITLE PAGE

Manuscript title: Cross-reaction of sera from COVID-19 patients with SARS-CoV assays

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1 **Abstract**

2 **Background:** The SARS-CoV-2 shares 74.5% genome identity with SARS-CoV, both
3 exhibiting a similar well conserved structure. Therefore, antibodies produced in COVID-19 and
4 SARS patients should not be that dissimilar. We evaluated SARS-CoV test assays to detect for
5 the presence of antibodies to SARS-CoV-2 and tried to determine the timing of appearance of
6 these antibodies by testing serial sera from these patients. **Methods:** Tests were carried out using
7 ELISA (total antibodies) and indirect immunofluorescence (IIFA) (IgM & IgG) methods on
8 serial sera from patients confirmed with SARS-CoV-2 infection. **Results:** Cross-reactivity was
9 seen in these two test assays with sera from COVID-19 patients and was detected in 6 out of 7
10 patients from 7 days after onset of symptoms. Five of the patients had detectable antibodies by
11 the 3rd week into their illness and there was evidence of seroconversion in 4 patients. The IIFA
12 method was marginally more sensitive compared to the ELISA assay, however the IIFA IgM test
13 was not useful in the early phase of the illness with poor sensitivity. **Conclusions:** Existing
14 diagnostic assays for SARS-CoV can detect antibodies in patients who were diagnosed with
15 COVID-19. These assays maybe be utilized as an interim measure in epidemiological
16 investigations for contact tracing and to determine the extent of community spread of this new
17 emerging virus pending the availability of specific serology tests for SARS-CoV-2.

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18 **Introduction**

19 SARS-CoV-2 is a new zoonotic coronavirus (CoV) that emerged in Wuhan, China which
20 was first reported on the 31st December 2019. As the number of cases of COVID-19 increases,
21 there is an urgent need to understand this outbreak that looks set to spread to several countries
22 around the world. The total number of cases is probably a gross underestimate, as patients may
23 present with the symptoms of the common cold, and therefore remain undiagnosed due to the
24 mild nature of this illness in the majority. Poorer resourced countries may also not have the
25 capability to equip themselves with complex molecular diagnostic setups and thus outbreaks in
26 those areas may go undetected for some time and the number of cases under reported.

27 The SARS-CoV-2 is a SARS-related virus with 74.5% genome identity to SARS-CoV.[1]
28 The similarities between these two viruses were described comprehensively in a recent published
29 article by Xu et al.[2] For structural proteins, including the nucleocapsid (N), matrix (M), and
30 envelope (E), high within-group conservation was maintained, with more modest similarity seen
31 across the entire CoV family. In contrast, the accessory proteins that distinguish CoV infections
32 from each other with high variability across the family, allow viruses to adopt to current and
33 novel hosts.[3] In a study which described the difference in amino acid substitutions of different
34 proteins for SARS-CoV-2 compared to SARS-CoV, it was found that there were no substitutions
35 that occurred in nonstructural protein 7 (nsp7), nsp13, envelop, matrix and accessory proteins p6
36 and 8b.[4] The N protein for SARS-CoV-2 has ~90% similar amino acid identity to the SAR-CoV
37 N protein and hence the SARS-CoV-2 antibodies against the N protein would likely recognize
38 and bind the SARS-CoV N protein as well.[5] Furthermore, a study by Zhou et al, showed that the

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39 SARS-CoV-2 could be cross-neutralized by horse anti-SARS-CoV serum at dilution 1:80,
40 confirming the relationship of the 2 viruses.[6]

41 Based on this knowledge, we postulated that the antibodies produced by COVID-19
42 patients should result in cross-reactivity to the SARS-CoV total antibody ELISA & IIFA tests
43 which utilizes whole SARS-CoV infected cells as the antigen substrate.

44 Patient's consent and ethical approval from the Ethics Committee were not required as
45 per the CIRB Research committee's guidelines and advice for the evaluation of this assay which
46 used existing anonymized human biological materials for test validation purposes and there is no
47 prospective collection of clinical, pathological and demographic information.

48 **Methods**

49 We identified SARS-CoV-2 positive cases which were confirmed by molecular testing of
50 respiratory specimens by real-time RT-PCR according to the published protocol by Corman et
51 al.[7] We retrieved residual samples left over from biochemical tests to obtain serial sera for these
52 patients. The Biochemistry department removed all patient identifiers and assigned random
53 numbers to each patient, and also included the number of days after onset of illness for each of
54 the retrieved specimens based on the information obtained from the Infectious Diseases team.
55 For negative controls, 10 samples which were sent for unrelated virology tests from 2 different
56 groups of patients were randomly selected. The first group consists of 5 sera collected 5 years
57 ago from our archive and the other group, another 5 sera from patients who were tested negative

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58 on two occasions for SARS-CoV-2 as part of the enhanced surveillance for patients who
59 presented with pneumonia but did not fulfil the criteria of suspected SARS-CoV-2 infection
60 during this outbreak period.

61 We performed two serological test methods on the selected samples using the SARS-CoV
62 total antibody ELISA test as described by Ksiazek et al[8] and Anti-SARS CoV Indirect
63 Immunofluorescence test (IIFT) (IgM & IgG) by Euroimmun (Germany) according to the
64 specified protocols and manufacturer's instructions respectively. Both these tests had previously
65 been validated by the authors and the manufacturer respectively, to have no cross reactivity with
66 antibodies from other known human coronaviruses.

67 **Results**

68 There were a total of 7 patients with confirmed COVID-19 admitted to our institution
69 during the study period. A total of 26 samples were retrieved from the Biochemistry department.
70 The number of samples obtained for each patient ranged from 1 – 9 (mean 3.7) with the earliest
71 taken 1 day after the onset of symptoms and latest at day 24. Five specimens were excluded due
72 to the narrow interval between samples or close proximity to the date of onset of illness. Figure 1
73 summarizes all the test results for these patients. Six out of the 7 patients had at least one positive
74 antibody result and seroconversion was demonstrated in 4 patients. The test results were negative
75 for all the negative control samples except for an IgM IIFT result which was deemed
76 indeterminate due to non-specific fluorescence. An example of a positive result by the IIFA
77 method for IgM and IgG in one of the samples is shown in Figure 2.

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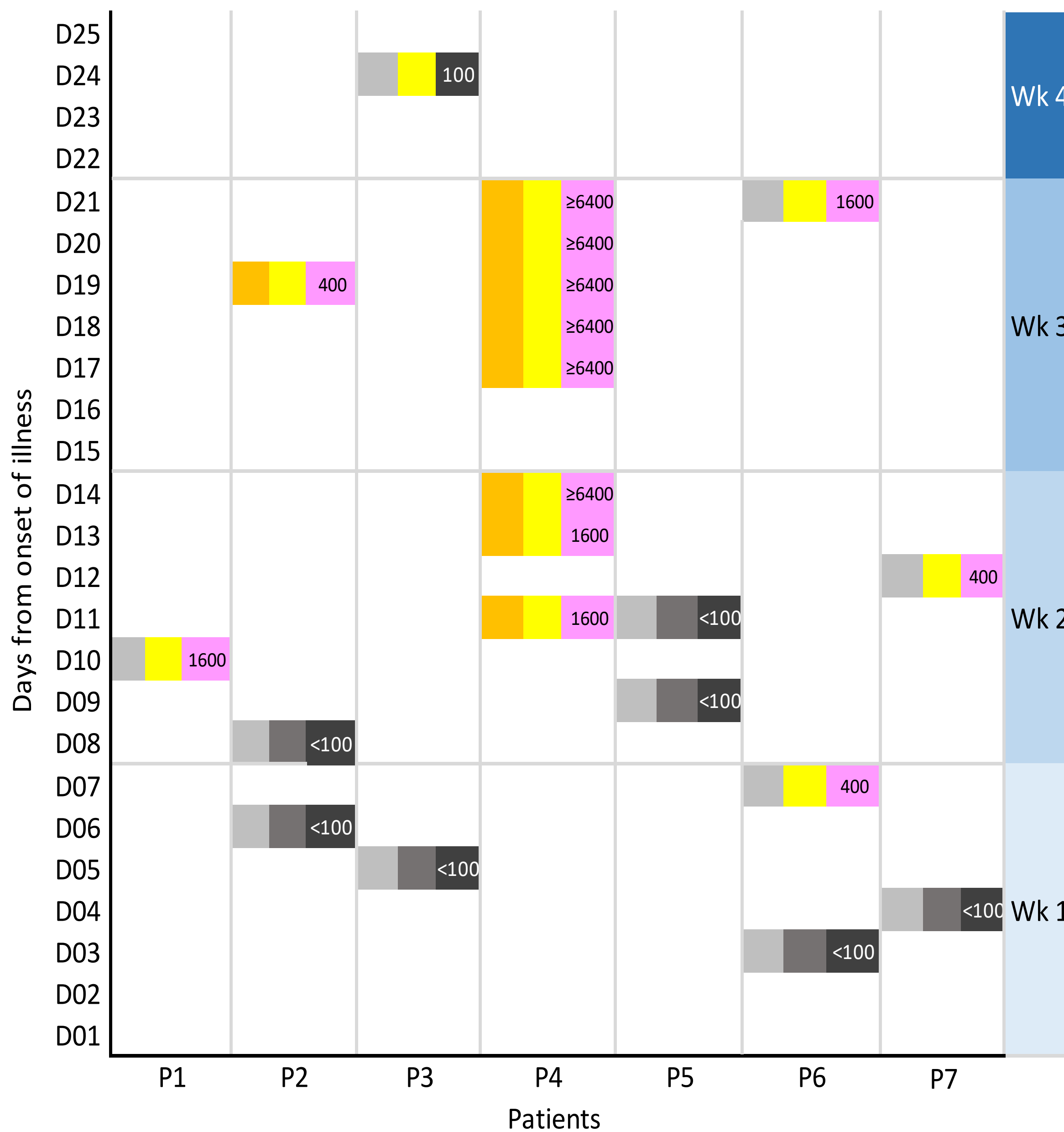
78 Figure 1: Test results for the COVID-19 patients



Key:

Pos IgM by IIFA

Neg IgM by IIFA

Pos IgG by IIFA

Neg IgG by IIFA

titer value* Pos total ab by ELISA (≥400)

titer value* Neg total ab by ELISA

*The value is reciprocal of the dilution (<100, 100, 400, 1600, ≥6400)

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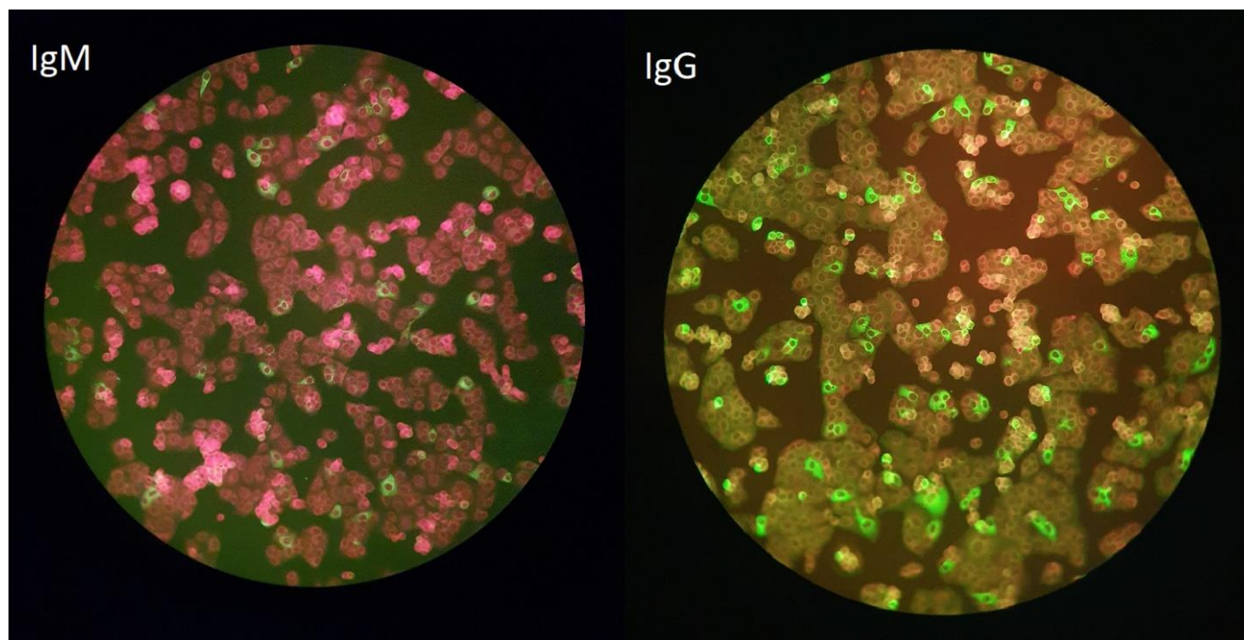
79 Figure 2: Example of a positive result by the IIFA method for IgM and IgG



80 **Discussion**

81 In SARS-CoV, both the IgM and IgG antibodies can appear as early as 1 week after
82 diagnosis in more than half of the patients in one study, with the IgM diminishing from week 5
83 to undetectable levels by week 11.[9] Other studies found that 80% of SARS-CoV patients were
84 antibody positive by 8 – 14 days after falling ill and the mean time to seroconversion was 20
85 days with 93% sensitivity of IgG detection by day 28.[10,11] Data from our SARS-CoV results of
86 140 tests by ELISA in 2003 showed that antibodies were positive in 15.4% of samples taken in
87 the 1st week of onset of symptoms, 46.5% in the 2nd week, 88.9% in the 3rd week and a 100%
88 after the 3rd week of onset of illness. For SARS-CoV-2, a study by Zhang et al which used an in-
89 house IgM and IgG ELISA test, found that 50% of their patients were positive for IgM from
90 samples taken on day 0 of hospital admission which increased to 81% by day 5, whereas positive

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91 IgG rates increased from 81% to 100%.[12] However these rates were based on the number of days
92 from the time of hospital admission rather than from the onset of clinical symptoms, hence the
93 early high proportion of positive antibody results reported is not representative of how soon the
94 IgM and IgG appears after infection in this study. In our current COVID-19 cohort (where sera
95 were available for analysis), 25% (1/4) of the patients had detectable antibodies in the 1st week of
96 illness, 66.7% (4/6) by the 2nd week and 100% (5/5) by the 3rd week of illness. These data are
97 somewhat imprecise due to the limited numbers of patients. In addition, we did not have samples
98 from P5 beyond the 2nd week for analysis.

99 Our evaluation study of both the ELISA and IIFA tests on SARS-CoV patients in 2003
100 showed that overall the IIFA test was 28.9% more sensitive than the ELISA test, which explains
101 the results of day 24 for P3. Although the overall results for the IIFA test may be more sensitive,
102 the IIFA IgM test in the COVID-19 patients was found to be less useful in the detection of acute
103 phase of the illness which is consistent with the findings of a study in SARS-CoV patients where
104 they found a less frequent (43%) and robust (less discriminatory) IgM response.[13] However, this
105 cannot be generalized as different assays will have different performances depending on the type
106 of antigen utilized. In most countries, real-time RT-PCR remains the diagnostic tool of choice in
107 the acute phase of infection given that antibody will take time to develop after the onset of
108 illness. These serological tests would be more useful in those who did not present early to a
109 healthcare facility to look for evidence of previous exposure to this virus. This is relevant for
110 contact tracing and to determine the true extent of the circulation of the virus to establish an
111 accurate case-fatality rate.

112 There is a possibility that positive antibodies from these tests could be as a result of
113 previous exposure to SARS 17 years ago. However, given that only 8,096 cases were reported

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114 worldwide and that the virus is not known to still be circulating in the community after it was
115 declared to be contained with no further reported cases in 2004 by WHO, this probability seems
116 very small and can be excluded by specific history taking.

117 Limitations of this study includes the relatively small number of patients and inconsistent
118 series of sera which ideally should have been collected at a predetermined regular time interval
119 to determine when IgM and IgG can be detected after infection in COVID-19 by these assays.
120 We also did not take into account other factors which could cause the delay in development of
121 antibodies such as immunosuppressive conditions and other treatment modalities that could
122 affect this. However, this study has provided evidence that antibodies to SARS-Cov-2 cross
123 reacts to give positive results in existing SARS-CoV test assays due to the similar structural
124 proteins that it shares with SARS-CoV. The positive predictive value of a serological test
125 depends on the prevalence of the virus and thus in the current situation where there is a
126 recognized outbreak, patients who present with recent compatible symptoms and test positive by
127 these tests are likely to have had exposure to SARS-CoV-2. Serological assays have the
128 advantage in terms of lower set-up costs, capacity for large volume processing, shorter
129 turnaround times, are less prone to specimen sampling quality issues, require lower specific
130 technical skills[14], have no risk of specimen contamination, involve handling of lower biohazard
131 risk specimen and expose healthcare workers to lower risk during sampling from patients
132 compared to molecular methods.

133 In conclusion, we provided proof of concept that the available SARS-CoV antibody
134 assays can reliably detect antibodies in patients with COVID-19 which could be used in this
135 current outbreak situation for serosurveys and as a diagnostic tool for under resourced countries.

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136 Further studies would be required to confirm their utility and better determine the time frame
137 when IgM and IgG is detectable in patients exposed to SARS-CoV-2.

138 **Acknowledgments**

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140 blinding of specimens, and the Infectious Disease team for providing onset date of illness which
141 was pertinent for the meaningful interpretation of results.

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