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1 **The Role of Antibody Testing for SARS-CoV-2: Is There One?**

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24 **Abstract**

25 The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)
26 brought with it rapid development of both molecular and serologic assays for identification of
27 COVID-19 infections. While Food and Drug Administration (FDA) emergency use authorization
28 (EUA) is required for clinical application of SARS-CoV-2 molecular tests, submission for EUA
29 is currently a voluntary process for manufacturers of serologic assays. The absence of FDA
30 oversight of serologic tests is concerning, given that the commercially available serologic assays
31 are highly variable, differing in their format, the antibody class detected, the targeted antigen and
32 the acceptable specimen types. An added complication is the lack of a clear understanding for
33 how such assays should be utilized and what the reported results ultimately indicate, or perhaps
34 more importantly, what they do not indicate. Here, we provide a brief summary of the
35 performance of a number of serologic assays reported in the literature, comment on what we do
36 and do not know regarding our immune response to SARS-CoV-2, and provide a number of
37 scenarios for which serologic testing will play a role in during our global response to this
38 pandemic.

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42 Shortly after its emergence in December 2019, the outbreak of severe acute respiratory
43 syndrome coronavirus 2 (SARS-CoV-2) was declared a pandemic in March 2020 by the World
44 Health Organization. A beta-coronavirus, SARS-CoV-2 is the seventh member of the
45 *Coronaviridae* family of viruses, and is the causative agent of coronavirus disease 2019
46 (COVID-19) in humans (1). Given the acute and rapid onset of COVID-19, molecular testing of
47 respiratory tract sample(s) to detect SARS-CoV-2 RNA remains the preferred diagnostic test for
48 assessment of symptomatic patients who meet COVID-19 testing criteria as defined by the
49 Centers for Disease Control and Prevention (CDC), and/or state and local health departments (2).
50 In addition to molecular testing, there is increasing interest for use of serologic assays to detect
51 antibodies against SARS-CoV-2. Unlike molecular testing, detection of an immune response to
52 the virus is an indirect marker of infection. As such, development of robust serologic tests,
53 alongside guidelines for appropriate utilization and interpretation relative to clinical and
54 epidemiological needs, is essential to maintain safe patient care standards and support ongoing
55 public health efforts.

56 Currently, over 91 manufacturers have notified the Food and Drug Administration (FDA)
57 that they are offering internally validated serologic tests for commercial use, and at the time of
58 this writing (April 17, 2020), four products have received FDA Emergency Use Authorization
59 (EUA) (3, 4). Unlike prior public health emergencies, the FDA has indicated that EUA is not
60 required for distribution or use of commercially available or laboratory developed SARS-CoV-2
61 serologic tests. Rather, they require that laboratories validate the assays as they deem appropriate
62 and notify the FDA of their use, alongside inclusion of specific report comments outlining the
63 limitations of these tests (3). The absence of FDA oversight of serologic tests is concerning given
64 that the commercially available serologic assays are highly variable, differing in their format

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65 (*e.g*., lateral flow immunoassays [LFAs], enzyme linked immunosorbent assays [ELISAs] and
66 chemiluminescent immunoassays [CLIA]), the antibody class(es) detected (*i.e.,* IgA, IgM, IgG,
67 or IgM/IgG total), the SARS-CoV-2 antigen(s) used to design the assay (*e.g.,* recombinant
68 nucleocapsid protein [NP], subunit 1 of the Spike glycoprotein [S1], the Spike glycoprotein
69 receptor binding domain [RBD], *etc*.), and the acceptable specimen type (*i.e.,* serum, plasma,
70 whole blood, finger stick whole blood). Given these differences in assay format and design, as
71 well as a dearth of peer-reviewed data on performance characteristics, it is critical that
72 laboratories considering serologic testing for SARS-CoV-2 perform a rigorous verification study
73 to ensure the analytical performance and clinical accuracy of test results.

74 Such validations must include assessment of specificity using samples collected prior to
75 or soon after the start of the outbreak from both healthy individuals and those with antibodies to
76 other common infectious pathogens and from non-infectious disease etiologies. Most concerns
77 regarding SARS-CoV-2 serologic assay specificity revolve around the potential for cross-
78 reactivity with antibodies to the commonly circulating alpha- (NL63 and 229E) and beta- (OC43
79 and HKU1) coronaviruses (CoVs). Prior seroprevalence studies indicate that over 90% of adults
80 age 50 and older have antibodies to all four common circulating CoVs, therefore the potential for
81 cross-reactivity in SARS-CoV-2 serologic assays is significant (5). Analysis of the amino acid
82 sequence homology for both the NP and S1 proteins, common antibody targets in commercially
83 available serologic tests, shows less than 30% similarity between the respective homologs found
84 in SARS-CoV-2 and the commonly circulating CoVs (6, 7). Although this in no way rules out
85 the potential for cross-reactivity, for comparison, SARS-CoV-2 and SARS share over 90%
86 homology at the amino acid level. Interestingly, recent preliminary studies by multiple groups
87 have shown limited to no cross-reactivity of antibodies to NL63, 229E, OC42 and HKU1

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88 coronaviruses against recombinant forms of SARS-CoV-2 NP and RBD proteins by Western blot
89 or ELISA analysis (7, 8). However, due to the absence of thorough specificity data, the FDA
90 currently requires inclusion of a comment indicating that false positive SARS-CoV-2 serologic
91 test results may occur in patients with antibodies to non-SARS-CoV-2 coronaviruses (3). With
92 respect to sensitivity studies, given our still emerging understanding of the kinetics of the
93 immune response and antibody dynamics against SARS-CoV-2, serologic test kits would ideally
94 be evaluated using serially collected sera from COVID-19 patients previously confirmed by a
95 molecular assay, or sera collected at a known time post-symptom onset (PSO). The resulting
96 information would allow laboratorians to provide clinicians preliminary guidance with respect to
97 timing of seroconversion relative to symptom onset, which due to the variety of serologic assays
98 available, may be specific to the particular test used in the laboratory.

99 As laboratorians consider the need for SARS-CoV-2 serologic testing, among the first
100 questions that likely arise are "How well do serologic tests for SARS-CoV-2 antibodies actually
101 work?" and "How will a SARS-CoV-2 serologic test result really be used in the clinical
102 practice?" Unfortunately, the answers to some of these questions remain challenging to define,
103 largely due to the limited peer-reviewed literature on serologic testing currently available.
104 Generally however, serologic assays are not relied upon for the diagnosis of acute viral
105 respiratory tract infections – the rapid disease onset, often prior to the development of an
106 immune response, and the availability of sensitive molecular diagnostics typically obviate
107 reliance on antibody testing. Recent studies have evaluated the potential role of IgM antibodies
108 against SARS-CoV-2 as a marker of recent infection. Among those, using an internally
109 developed ELISA with recombinant SARS-CoV-2 NP antigen, Guo and colleagues recently
110 showed that IgM antibodies were detectable in 85% of COVID-19 confirmed patients 1 to 7 days

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111 PSO (7). Importantly however, they state that molecular testing remains preferred, with higher
112 sensitivity during the first 5.5 days after illness onset, and conclude that IgM against SARS-
113 CoV-2 may be useful in suspected COVID-19 patients negative by molecular methods after this
114 time point. In stark contrast, albeit not yet peer-reviewed, another study evaluating a magnetic
115 CLIA against the same NP antigen, showed 12% to 40% IgM seroconversion during the same
116 timeframe post onset (9). Using an ELISA designed to detect IgM antibodies against the RBD of
117 the S1 subunit of the SARS-CoV-2 spike glycoprotein, data from Zhao *et. al* indicate that only
118 approximately 28% of patients seroconvert to IgM positive by day 7 PSO, whereas 73% are
119 positive by day 14 (10). In addition to IgM-based SARS-CoV-2 serologic assays, at least one
120 immunologic assay to detect IgA-class antibodies against SARS-CoV-2 is also commercially
121 available. IgA antibodies are the most abundant immunoglobulins in mucosal surfaces, playing
122 an essential role in protective immunity via toxin and viral neutralizing activities in the
123 respiratory and gastrointestinal tracts (11, 12). Similar to IgM, recent studies show that IgA
124 antibodies against SARS-CoV-2 are detectable as early as one day after symptom onset (7). The
125 specificity of IgA-based assays have not yet been well vetted in the literature however. To date, a
126 pre-print study concluded that despite higher sensitivity soon after infection, IgA specificity was
127 lower compared to IgG-based tests, an observation that has been mirrored in unpublished studies
128 by an author of this commentary (E.S. Theel, P. Slev and S. Wheeler, unpublished data) (6).
129 Finally, assessment of IgM and IgA antibody responses in patients infected with SARS virus
130 showed that these two antibody classes did not provide earlier evidence of infection compared to
131 IgG antibody testing (13). Collectively, the data presented in these initial studies and prior
132 findings with SARS, suggest that results from SARS-CoV-2 IgM and IgA serologic tests, if

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133 used, should be interpreted with significant caution until more robust performance characteristic
134 and utilization studies are available.

135 In contrast to IgM and IgA class antibodies, detection of IgG antibodies against SARS-
136 CoV-2 may have a larger role to play during this pandemic. Compared with other antibody
137 classes, IgG is a longer lasting antibody and similar to IgA, is associated with viral neutralizing
138 activity, which is likely essential for recovery from COVID-19 (11, 14). Preliminary data suggest
139 that IgG developed against different SARS-CoV-2 antigens becomes detectable in
140 immunocompetent patients after at least 8 days PSO, with over 90% of individuals seropositive
141 after day 14 of illness, although some individuals may take longer to seroconvert depending on
142 their immune status, or may never seroconvert if significantly immunosuppressed (9, 10).
143 Although limited in breadth and not all yet peer-reviewed, initial studies suggest fairly high
144 specificity (>95%) for IgG-based SARS-CoV-2 serologic assays against commonly circulating
145 coronaviruses and other infectious pathogens (8, 9). Also, according to one reputable ELISA
146 manufacturer, the false positivity rate observed with their SARS-CoV-2 S1-based IgG ELISA
147 was 2.5% in sera positive for a diverse range of autoantibodies and 3.4% in sera from influenza
148 vaccine recipients – such antibodies are not uncommon in the US population. Importantly, true
149 specificity studies require head-to-head comparison of commercially available serologic assays
150 with neutralizing antibody tests, which are not widely accessible given the challenges of
151 performing such assays. Currently, all available IgG serologic assays for SARS-CoV-2 are either
152 qualitative or semi-quantitative in design. For well-vetted assays, a negative result may indicate
153 either no prior exposure or, for samples collected too soon after illness onset or from
154 immunosuppressed patients, the absence of an as of yet detectable immune response. In contrast,
155 a positive SARS-CoV-2 IgG result implies infection with the virus at some point in the recent or

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156 remote past. Importantly, however, the presence of SARS-CoV-2 IgG does not equate to
157 protective immunity against re-infection nor does it indicate whether a patient has stopped
158 shedding virus. In theory,seropositive individuals are expected to be at lower risk for re-infection
159 compared to seronegative persons, however neither the level nor the duration of protective
160 immunity against COVID-19 is currently known. The potential for at least short term immunity
161 to COVID-19 is not unfounded however. From prior immunity studies in recovered SARS
162 patients, we know that neutralizing antibodies were detectable in 89% of patients up to 2 years
163 after infection, with IgG antibodies becoming undetectable at 6 years (15, 16). Additionally,
164 although not yet peer-reviewed, preliminary SARS-CoV-2 challenge studies in COVID-19
165 recovered adult rhesus macaques suggest that primary infection leads to protective immunity for
166 at least one month post recovery (17). The true temporal duration of protective immunity to
167 COVID-19, partial or otherwise, will take time to establish.

168 The reference standard method for detection of neutralizing antibodies, which may be
169 used as a correlate of protective immunity, remains plaque reduction neutralization tests
170 (PRNTs). These tests are not routinely performed in clinical laboratories however, as they
171 involve live viral culture, which for SARS-CoV-2 requires biosafety level 3 (BSL3) containment
172 facilities, are laborious, dependent on a high level of expertise and are not amenable to
173 automation. Although alternative BSL2 protocols using pseudotyped Vesicular Stomatitis Virus
174 (VSV) expressing different SARS-CoV-2 surface antigens are being developed to obviate the
175 need for culture of live SARS-CoV-2, these methods remain in the research arena (18).
176 Importantly, regardless of which neutralizing antibody test is being performed, it remains unclear
177 what minimal neutralizing antibody titer correlates with protective immunity and whether results
178 from the commercially available SARS-CoV-2 serologic assays can predict such immunity.

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179 Despite these significant unknowns, there remains interest and even demand to perform serologic
180 tests at a national scale, with the potential to make consequential decisions based on the reported
181 results.

182 The following are scenarios for which SARS-CoV-2 serologic testing, specifically IgG-
183 based assays, may be useful given our current knowledge of the virus, our limited understanding
184 of the immune response to it, and the urgent need for improved antiviral therapies and preventive
185 measures.

186 **Screening of Recovered COVID-19 Patients for Convalescent Plasma Therapy.** Currently,
187 among the most advocated patient-centered use of SARS-CoV-2 serologic testing is for
188 screening of COVID-19 recovered patients for the presence of anti-SARS-CoV-2 antibodies. If
189 present, COVID-19 convalescent plasma (CCP) collected from these donors may be used to treat
190 acutely ill patients with COVID-19 (19). Clinical trials are currently on-going across the nation
191 to evaluate the efficacy of convalescent plasma therapy in both sick patients and as potential
192 post-exposure prophylaxis of health care workers (HCWs; www.ccpp19.org). Notably, the FDA
193 investigational drug use (IND) requirements for these clinical trials, or for emergency IND use,
194 indicate that donor convalescent plasma should have a neutralizing antibody titer of at least
195 1:160, although a titer of 1:80 is acceptable in the absence of other plasma
196 (https://www.fda.gov/vaccines-blood-biologics/investigational-new-drug-ind-or-device-
197 exemption-ide-process-cber/recommendations-investigational-covid-19-convalescent-plasma;
198 accessed 4/11/2020). Unfortunately, neutralizing antibody tests are not widely available and
199 results from commercially available serologic assays are not known to correlate to neutralizing
200 antibody titers. Given the urgent need of convalescent plasma as potential bridging therapy until
201 more targeted treatments or preventative measures are available, validated SARS-CoV-2 IgG

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202 serologic assays may be used to rapidly screen potential donors for the presence or absence of
203 antibodies, with the goal of subsequently testing positive samples by neutralization assays.
204 Studies are also ongoing to determine whether the semi-quantitative results from a number of
205 SARS-CoV-2 IgG ELISAs show any correlation to neutralizing antibody levels. Notably, a
206 recent study on this topic showed poor correlation between a spike protein-based IgG serological
207 test and PRNT, suggesting that such a correlative approach between currently available
208 commercial assays and neutralizing antibody titers may not be possible (6, 20).

209 **SARS-CoV-2 Seroprevalence Studies.** Serologic testing to detect IgG-class antibodies against
210 SARS-CoV-2 will play an essential role in determining the true prevalence of this virus. This is
211 particularly true if one considers the constant discussions around positive and negative predictive
212 values of molecular tests for SARS-CoV-2. A prevalence of total disease in the community
213 needs to be established in order to perform such calculations with any meaning. Given that the
214 rate of asymptomatic infection with SARS-CoV-2 continues to be refined, with previously
215 reported rates ranging from 4% to 80% across different populations and exposure scenarios, such
216 seroprevalence studies will allow us to establish a more accurate regional or national
217 denominator for the number of infected individuals, which will ultimately help to determine a
218 true case fatality rate. (21-23) (https://www.who.int/news-room/q-a-detail/q-a-similarities-and-
219 differences-covid-19-and-influenza; accessed 4/12/2020). Importantly however, the serologic
220 assay(s) utilized for such seroprevalence studies must exhibit exceptionally high specificity (≥
221 97%) given that the prevalence of SARS-CoV-2 infection in the United States is likely still fairly
222 low and the potential impact of cross-reactive antibodies to other circulating CoVs – a test with
223 lower specificity could create significant bias and high rates of false positive results in large
224 scale sero-surveys. Carefully-designed serial seroprevalence studies, performed over time and

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225 including large cohorts will also provide us with a better understanding of transmission patterns
226 and may help determine when (or if) we reach a state of herd immunity. Herd (population)
227 immunity, occurs when a sufficient proportion of the population becomes immune to the
228 infectious agent, thus limiting the chance for further infections to occur. The percentage of
229 individuals that must be immune for this to occur depends on multiple factors, including the
230 infectiousness or transmissibility of the infectious agent – the more transmissible the agent, the
231 higher percentage of the population that needs to be immune for herd immunity to be effective.
232 The precise threshold for what percentage of the population would need to be immune to SARS-
233 CoV-2 for this to occur is currently undefined, however assuming that the SARS-CoV-2 basic
234 reproductive number (R_0) ranges from 2 to 3.5, this threshold may range from 40% to 75% (24).
235 It is paramount to note however, that given the early and intense social distancing measures
236 instituted by federal and local governments, viral transmission has likely significantly decreased,
237 to the point that the actual herd immunity may not be achieved until such public health measures
238 are lifted. Once available, a safe and efficacious vaccine should be able to induce widespread,
239 population-level immunity.

240 **Monitoring Immune Responses to Candidate COVID-19 Vaccine Candidates**. The most
241 recent reports indicate that there are over 100 SARS-CoV-2 vaccine candidates either in
242 development, in initial preclinical stages, or which have entered human clinical trials (25). At
243 least five of these are currently in Phase 1 clinical trials and vary in their design, ranging from
244 the use of lipid nanoparticles expressing the SARS-CoV-2 spike glycoprotein to modified
245 dendritic cells expressing synthetic mini-genes from selected viral proteins. Serologic testing for
246 SARS-CoV-2 will play an important role for pre-screening individuals prior to admission into
247 vaccine clinical trials, and to monitor the temporal immune responses in vaccine recipients and

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248 ultimately help to define vaccine efficacy. It is important to note that serological assays able to
249 detect a neutralizing antibody response, (*i.e.,* PRNT) will be critical to provide the most accurate
250 results for vaccine immunogenicity trials. Notably, whether such antibodies would potentially
251 mediate antibody-dependent enhancement leading to adverse events is an important question that
252 will be addressed through efficacy trials and post-vaccine surveillance.

253 **Summary**

254 As a result of the novelty of SARS-CoV-2 and the limited data currently available
255 regarding our immune response to it, well vetted utilization strategies for SARS-CoV-2 serologic
256 assays are lacking. Use of anti-SARS-CoV-2 antibody tests performed at a population-level to
257 guide return-to-work decisions or to 're-start the economy' is a topic of widespread discussion at
258 the local, state and national levels. Undeniably, this is an intriguing concept, with mass serologic
259 screening potentially achievable at a national scale. However, we must remain cognizant of the
260 current challenges and limitations of such an approach. First, there remains significant concern
261 among laboratorians with respect to the over 91 serologic tests that are currently commercially
262 available, for which the performance characteristics are not yet known. In fact, reports of poorly
263 performing serologic tests are already emerging in the media
264 (https://www.cnn.com/2020/04/05/health/coronavirus-infection-tests/index.html; accessed April
265 12, 2020). Should mass screening be recommended at the state or national level, it is imperative
266 that data-based guidance regarding serologic test accuracy is available to laboratories
267 considering such testing. Second, as outlined above, although a positive SARS-CoV-2 IgG result
268 suggests prior infection with the virus, it does not independently imply protective immunity.
269 Similarly, the duration of such immunity remains unknown. Finally, depending on the timing of
270 SARS-CoV-2 infection and sampling for serologic testing, recently infected individuals may be

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271 IgG positive, yet still be shedding virus as determined by molecular assays. Whether the detected
272 viral RNA in these individuals equates to transmissible virus cannot be resolved without viral
273 culture of the specimen at BSL-3 containment – a method not available in clinical laboratories.
274 Notably, a recent small study in hospitalized patients showed that infectious virus was not
275 detectable in culture from seroconverted patients 8 days after of symptom onset, whereas
276 molecular testing of nasopharyngeal swab specimens remained positive beyond 14 days for most
277 patients, suggesting that detected RNA by these assays represents residual RNA from non-
278 infectious virus (20). This study however, was conducted using mildly symptomatic individuals.
279 Given that severely-ill individuals remain SARS-CoV-2 RNA positive for several weeks despite
280 the appearance of neutralizing antibodies, further studies using viral culture are necessary to
281 better determine the period of transmissibility (26).

282 In conclusion, the availability of serologic assays to detect antibodies against SARS-
283 CoV-2 presents us with additional tools to use from our SARS-CoV-2 pandemic response
284 toolbox. As we learn more about our immune response to SARS-CoV-2, its level and duration of
285 protective immunity, and as we gain a better understanding of the advantages and limitations of
286 commercially available serologic assays, more defined, patient-centered utilization guidelines
287 will likely emerge. These tests may be useful from a public health, risk management, and
288 academic perspective, but additional data is required to fully drive this response.

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