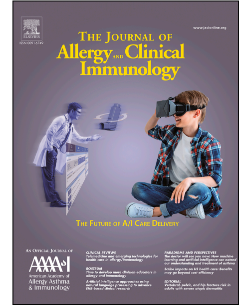
Journal Pre-proof



SARS-CoV-2 Antibody Testing – Questions to be asked

Mustafa K. Özçürümez, MD, Andreas Ambrosch, MD, Oliver Frey, MD, Verena Haselmann, MD, Stefan Holdenrieder, MD, Michael Kiehntopf, MD, Michael Neumaier, MD, Michael Walter, MD, Folker Wenzel, MD, Roman Wölfel, MD, Harald Renz, MD, on behalf of the COVID-19 Task Force of theGerman Society for Clinical Chemistry and Laboratory Medicine (DGKL)

PII: S0091-6749(20)30739-9

DOI: https://doi.org/10.1016/j.jaci.2020.05.020

Reference: YMAI 14586

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 30 April 2020

Revised Date: 18 May 2020

Accepted Date: 25 May 2020

Please cite this article as: Özçürümez MK, Ambrosch A, Frey O, Haselmann V, Holdenrieder S, Kiehntopf M, Neumaier M, Walter M, Wenzel F, Wölfel R, Renz H, on behalf of the COVID-19 Task Force of theGerman Society for Clinical Chemistry and Laboratory Medicine (DGKL), SARS-CoV-2 Antibody Testing – Questions to be asked, *Journal of Allergy and Clinical Immunology* (2020), doi: https://doi.org/10.1016/j.jaci.2020.05.020.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2020 American Academy of Allergy, Asthma & Immunology

Journal Pre-proof

1 # SARS-CoV-2 Antibody Testing – Questions to be asked

2 **Mustafa K. Özçürümez MD[1], Andreas Ambrosch MD[2*], Oliver Frey MD[3,4*],**

3 **Verena Haselmann MD[5*], Stefan Holdenrieder MD[6*], Michael Kiehntopf MD[7*],**

4 **Michael Neumaier MD[5*], Michael Walter MD[8,9*], Folker Wenzel MD[10*],**

5 **Roman Wölfel MD[11,12*], Harald Renz MD[13*];**

6 **on behalf of the COVID-19 Task Force of the**

7 **German Society for Clinical Chemistry and Laboratory Medicine (DGKL).**

8 *Co-Authors are listed in alphabetical order

9 (1) Department of Laboratory Medicine of the Medical Clinic at the University
10 Medical Center Knappschaftskrankenhaus Bochum, Ruhr University, Bochum,
11 Germany;

12 (2) Institute of Laboratory Medicine, Microbiology and Hygiene, Barmherzige
13 Brüder Regensburg, Germany;

14 (3) Institute of Laboratory Medicine, Brandenburg Medical School, Brandenburg an
15 der Havel, Germany;

16 (4) Institute of Medical Diagnostics, Berlin, Germany;

17 (5) Institute of Clinical Chemistry, University Medicine Mannheim, Medical Faculty
18 Mannheim of the University of Heidelberg, Mannheim, Germany;

19 (6) Institute for Laboratory Medicine, German Heart Centre, Technical University
20 Munich, Munich, Germany;

21 (6) Institute of Clinical Chemistry, University Medicine Mannheim, Medical Faculty
22 Mannheim of the University of Heidelberg, Mannheim, Germany;

Journal Pre-proof

Journal Pre-proof

23 (7) Department of Clinical Chemistry and Laboratory Medicine, Jena University

24 Hospital, Jena;

Journal Pre-proof

Journal Pre-proof

25 (8) Institute of Clinical Chemistry and Laboratory Medicine; Rostock University
26 Medical Center; Rostock, Germany;

27 (9) Institute of Laboratory Medicine, Clinical Chemistry, and Pathobiochemistry,
28 Charité University Medical Center; Berlin, Germany;

29 (10) Faculty of Medical and Life Sciences, Hochschule Furtwangen, Germany;

30 (11) Bundeswehr Institute of Microbiology, Munich, Germany;

31 (12) German Center for Infection Research (DZIF), Partner Site Munich, Germany;

32 (13) Institute of Laboratory Medicine, Universities of Giessen and Marburg Lung
33 Center (UGMLC), Philipps Universität Marburg, German Center for Lung
34 Research (DZL) Marburg.

35 **CORRESPONDING ADDRESS**

36 Mustafa K. Özçürümez, MD
37 Department of Laboratory Medicine of the Medical Clinic at the University Medical
38 Center Knappschaftskrankenhaus Bochum, Ruhr University, In der Schornau 23-25,
39 44892 Bochum, Germany. Phone (+49) 234 299-3401; Fax (+49) 234 299-3409;
40 e-mail: mustafa@ozcurumez.de

41 **FUNDING**

42 Harald Renz is funded by the Universities Giessen Marburg Lung Center and the
43 German Center for Lung Disease (DZL German Lung Center, no. 82DZL00502) for
44 UGMLC

45 **CONFLICTS OF INTEREST**

46 All authors declare no conflicts of interest.

Journal Pre-proof

Journal Pre-proof

47 **TOTAL WORD COUNT**

48 4710 words

Journal Pre-proof

Journal Pre-proof

49 **ABSTRACT**

50 SARS-CoV-2 infection and development of COVID-19 disease presents a major
51 healthcare challenge of global dimensions. Laboratory diagnostics of infected
52 patients, and the assessment of immunity against the SARS-CoV-2 virus presents a
53 major cornerstone in handling the pandemic. Currently there is an increase in
54 demand of antibody testing and a large number of tests are already marketed or in
55 the late stage of development. However, the interpretation of test results depends on
56 many variables and factors, including sensitivity, specificity, potential cross-reactivity
57 and cross-protectivity; the diagnostic value of antibodies of different isotypes, the use
58 of antibody testing in identification of acutely ill patients or in epidemiological settings.
59 In this article the recently established COVID-19 Task Force of the German Society
60 for Clinical Chemistry and Laboratory Medicine (DGKL) addresses these issues
61 based on the currently available datasets in this rapidly moving field.

62 **KEYWORDS**

63 Antibody response; COVID-19; diagnostic pathway; external quality assurance;
64 immunity; immunoassay; neutralization assay; respiratory infections; serologic
65 analysis; severe acute respiratory syndrome coronavirus 2.

Journal Pre-proof

Journal Pre-proof

66 **ABBREVIATIONS**

67 ARDS, adult respiratory distress syndrome; BSL, biosafety level; CE, Conformitè
68 Europëenne;++ COVID-19, coronavirus disease 2019; ECDC, European Centre for
69 Disease Prevention and Control; EIA, enzyme immunoassay; ELISA Enzyme-linked
70 Immunosorbent Assay; EQA, external quality assessment; EU, European Union;
71 EUA, emergency use authorization; FIND, Foundation of Innovative New
72 Diagnostics; HCoV, Human Coronavirus; HCoV-OC43, Human Coronavirus OC43;
73 IVD, in-vitro diagnostics; MERS, Middle East Respiratory Syndrome Coronavirus;
74 NPV, negative predictive value; PPV, positive predictive value; RBD, receptor binding
75 domain; RfB, Referenzinstitut für Bioanalytik; RT-PCR, Reverse transcription
76 polymerase chain reaction; SARS, severe acute respiratory syndrome; SARS-CoV-2,
77 severe acute respiratory syndrome coronavirus 2; SEIR, susceptible-exposed-
78 infected-resistant; UK-NEQAS, United Kingdom National External Quality
79 Assessment Service; WHO, World Health Organization.

Journal Pre-proof

Journal Pre-proof

80 **INTRODUCTION**

81 The infection with the SARS-CoV-2 coronavirus and the development of COVID-19
82 disease represents a major healthcare challenge of global dimensions. The current
83 SARS-CoV-2 pandemic fells partly like a reminiscence of the earlier SARS epidemic
84 in 2002/2003. Only in part because similar requirements and developments in
85 diagnostics were necessary and similar challenges existed with regard to the
86 evaluation of test results[1]. A major difference to that time is the strong political and
87 economic pressure to insist on the most reliable high-throughput diagnostics. There
88 is an urgent need for the development of appropriate laboratory tests to identify
89 infected patients, follow the course of viral shedding and clearance and to assess
90 immunity against SARS-CoV-2. Laboratory testing is built on two different pillars: on
91 the one side, the detection and measurement of viral RNA, and on the other side
92 measuring antibodies of various isotypes against SARS-CoV-2 components,
93 reflecting the host immune response. Although antibodies are developing quite early
94 during the course of the disease, the serological response is not suitable for early
95 detection of infected patients. Furthermore, the clinical and immunological meaning
96 of these antibody responses is unclear, since the many available tests do not
97 necessarily prove protective immunity against the SARS-CoV-2 virus. Furthermore, it
98 can be questioned, whether serological testing can be used as a surrogate marker
99 for viral encounter. In this regard it remains unclear whether oligo- or
100 monosymptomatic cases – which are still the majority of all SARS-CoV-2 infected
101 patients – also develop this type of immune response. In addition, the longevity of the
102 persistence of these antibodies is still not clear. There is increasing interest to use
103 antibody testing to assess the immune status of larger populations and also of the
104 risk population such as healthcare workers and others, in order to help to draw

Journal Pre-proof

Journal Pre-proof

105 conclusions from drastic measures such as economic and social lockdown, social
106 distancing and other restrictive actions. These key questions require immediate
107 attention, in order to appreciate the strength and weakness of antibody testing
108 against SARS-CoV-2. This article summarizes the currently available knowledge and
109 literature in this extremely rapidly moving area.

110 **MAIN TEXT**

111 **What are the approved indications to perform a COVID-19 serology?**

112 In most patients, antibodies against SARS-CoV-2 become detectable within the first
113 10 days after onset of symptoms of COVID-19. Also the kinetics of the class switch of
114 different isotypes of SARS-CoV-2 specific immunoglobulins is comparable to other
115 coronavirus infections[2–10]. IgM, IgA and IgG antibodies were detectable in some
116 patients as early as day 1 after onset of symptoms. The interquartile ranges of the
117 first antibody detection for IgM and IgA are between day 3 and 6, and for IgG
118 between day 10 and 18. IgA reached a plateau up to day 7, while IgM and IgG
119 continuously increased until day 14 and day 21, respectively[5]. Therefore, serological
120 testing could be useful in several different aspects of COVID-19[11].

121 First, and perhaps most important, serological testing could supplement standard RT-
122 PCR assays for diagnosis of COVID-19 in symptomatic patients. There is
123 accumulating evidence that viral shedding in the upper respiratory system profoundly
124 decreases 7-10 days after infection, leading to negative swab results in at least 30-50
125 percent of COVID-19 cases[6,12–14]. Measurement of SARS-CoV-2-specific antibodies,
126 which begin to be detectable in a significant proportion of patients 5-7 days after
127 infection and later in almost all cases, could help to detect cases with negative RT-

Journal Pre-proof

Journal Pre-proof

128 PCR tests[5,15]. However, antibody tests will not replace direct pathogen detection
129 since the immunological response triggered by an acute infection like COVID-19 has
130 a certain latency.

131 Second, serological testing is considered to be used to retrospectively determine
132 SARS-CoV-2 infections in people that previously have not tested positive by RT-PCR
133 for whatever reasons. However, the kinetics and the magnitude of the antibody
134 response seems to correlate with the clinical severity of the disease[4,5]. Preliminary
135 data suggest that an yet unknown number of asymptomatic infected and even
136 oligosymptomatic COVID-19 patients do not develop seroconversion[16,17].

137 There is a lack of validation data from IVD manufacturers who have systematically
138 examined asymptomatically infected patients. Therefore, it is currently challenging to
139 establish cut-off values that are sensitive enough to determine the prevalence of
140 infection at the population level without running the risk of too high rates of false-
141 positive results. Performance data about the Roche antibody assay have been
142 currently released[18]. The assay exhibited no cross-reactivity with 40 endemic HCoV
143 convalescence sera, i.e. it yieled a specificity of 100% (95% confidence interval
144 91.2% to 100%). More striking, among 5272 pre-CoViD-19 sera collected from
145 routine labs (n = 3420) and blood donors (n = 1772) only 10 reactive sera were
146 identified, i.e. a specificity of 99.81% (95% confidence interval 99.65% to 99.91%)
147 was achieved. With increasing knowledge about SARS-CoV-2, the problem of
148 specificity could fade into the background in the future and the use of serology as an
149 epidemiological instrument become the next challenge.

150 Third, and of utmost importance for the healthcare system and political decisions on
151 lock down measures, is the ability of serological testing to establish indicators of
152 protection against (re-) infection with SARS-CoV-2. Indeed, sera from patients with

Journal Pre-proof

Journal Pre-proof

153 COVID-19 show neutralizing activity in vitro and recently published case series on
154 plasma transfer from convalescent COVID-19 patients demonstrate also in vivo
155 effects[4,19–21]. However, the efficacy of this therapy has not yet been confirmed in
156 sufficiently large, controlled studies. Furthermore, no direct conclusion can be drawn
157 about a reliable protective effect of the antibodies individually acquired during an
158 infection. It is therefore conceivable that anti-SARS-CoV-2 antibodies can protect
159 against the virus. However, demonstrating a neutralizing activity of an antibody
160 against a virus requires assays using live or pseudotyped virus, which cannot be
161 performed in a high-throughput fashion. It is necessary to determine the targets of
162 protective antibodies in order to develop simple immunoassays that best reflect virus
163 neutralization. This is especially important since certain target epitopes of antibodies
164 might also enhance virus entry[22]. Therefore, total antibody measurements do not
165 necessarily reflect protection after infection, nor do they indicate the efficacy of a
166 vaccination to ascertain immunity.

167 **How valuable is SARS-CoV-2 antibody testing in diagnostic pathways?**

168 In a cross-validation of 22 assays (lateral flow tests and ELISAs) to detect IgM and
169 IgG antibodies in COVID-19 patients, a significant number of positive results were
170 also found in historic sera from the pre-COVID-19 era and from non-SARS-CoV-2
171 infections[23,24] resulting in test specificities ranging from 84% to 100% for both
172 isotypes (95% confidence intervals 76% to 91% and 97% to 100%, respectively). The
173 reported specificity of 100% for both, IgG and IgM, was yielded by one of the lateral
174 flow assays, however, especially evident for IgM, sensitivity within the first 10 days
175 after patient reported symptom onset was lower as compared to the other assays.

Journal Pre-proof

Journal Pre-proof

176 In case of a positive test result, the prevalence of the disease at the population level
177 is the main determinant of the positive predictive value (PPV). The recently reported
178 prevalence of COVID-19 in the population[25,26] of 1 % to 4 % will result in a PPV
179 between 25% and 58% assuming a specificity of 97% and between 4% and 15% for
180 76% specificity, respectively, at an artificial sensitivity of 100% in all scenarios It is
181 therefore not possible to infer protection against SARS-CoV-2 from a positive result
182 of an immunoassay (see Figure 1).

183 Figure 2 shows an example of PPV / NPV values (y-axis) as a function of prevalence
184 (x-axis) for theoretically assumed test sensitivities/ and specificities from 80% to
185 99.9%, respectively, and for two commercially available SARS-CoV-2 IgG tests with
186 sensitivities of 88.66% and 80% and specificities of 90.63% and 98.5%, respectively.

187 Applying these assay performance figures to testing strategies in the general
188 population, predictive values of 2.2% to 7.9% (PPV) and 99.97% to 99.89% (NPV) or
189 11.4% to 32.6% (PPV) and 99.95 to 99.82% (NPV) can be calculated for a
190 prevalence of 0.24% (Regensburg, Bavaria, Germany) or 0.9%[26], respectively.
191 Clinical triage for COVID-19 symptoms increases the pre-test probabilities towards
192 48% in hospitalized settings and will raise the PPV for the same tests to 89.73% and
193 98.01% while NPV slightly decreases to 89.65% and 84.33%, respectively.

194 In the latter case, patients were questioned about COVID-19 symptoms when
195 admitted to the emergency center, and only tested in cases of abnormalities
196 (Rockmann & Ambrosch, personal communication 2020). From the exemplary
197 calculations of PPV / NPV with known sensitivity / specificity and different
198 prevalence, it thus becomes clear under which basic conditions and prerequisites
199 (pre-test probability) a serological test can basically be carried out and interpreted
200 sensibly.

Journal Pre-proof

Journal Pre-proof

201 The dynamics of the respective antibody classes (IgA / IgM versus IgG) in the course
202 of the infection and their dependence on the severity of the infection represent
203 additional factors which contribute significantly to the indication and interpretation of
204 results for serological antibody testing. While in infections with clear respiratory
205 symptoms caused by SARS-CoV-2, only 50 % of the seroconversion seems to occur
206 on day 7 after the onset of symptoms (IgA / IgG or IgM / IgG), SARS-CoV-2 spike
207 protein as antigen[26–28] and is completed on day 14, in severe cases of ARDS
208 seroconversion seems to occur earlier[4]; in mild or asymptomatic cases
209 seroconversion may even be absent[26].

210 **Do SARS-CoV-2 specific antibodies indicate the end of infectivity?**

211 The detection of persistent infectivity cannot be conclusively verified by commercially
212 available RT-PCR because it is not possible to distinguish between replicable virus
213 components and inert genome fragments. It is therefore assumed that RT-PCR
214 results lag behind the actual elimination of SARS-CoV-2 in infected individuals.

215 The virological gold standard to prove infectivity is virus isolation in cell culture[13]. In
216 addition, novel molecular methods for detection of subgenomic RNA can be used to
217 prove the end of active replication of SARS-CoV-1[29] and also SARS-CoV-2 in
218 infected cells[14]. In general, innate and adaptive defense mechanisms are involved in
219 virus elimination and prevent further infections. As yet, only limited data are available
220 on antibody responses during SARS-CoV-2 infection. Looking at the course of the
221 virus load in COVID-19 IgA and IgM antibodies, seroconversion is not accompanied
222 by an abrupt elimination of SARS-CoV-2. Rather, a slow but steady decrease of the
223 viral load in the sputum coincides with the course of seroconversion at the beginning
224 of week two[14,30]. At this time, there is no sufficient evidence to conclude that the

Journal Pre-proof

225 detection of SARS-CoV-2 specific antibodies can be linked to the end of the virus'
226 infectivity. Further studies are needed to better understand the role of the various
227 types of antibodies for different disease courses of COVID-19.

228 **What does the detection of neutralizing antibodies imply about the immunity**
229 **against SARS-CoV-2?**

230 SARS-CoV-2 targets the mucous membranes and induces the release of secretory
231 IgA within the first week of symptoms, followed by IgM and IgG in the second week.
232 As with SARS and MERS, IgM cannot be detected significantly earlier than IgG[8].
233 Those antibodies which bind specifically to surface structures of SARS-CoV-2, like
234 the S protein, prevent the virus from interacting with its target cell are called
235 neutralizing antibodies. These antibodies play an important role in virus clearance as
236 they have the ability to block viral infection and are assumed to protect patients.
237 Serological tests for SARS-CoV-2 that are intended to confirm such neutralizing
238 antibodies must therefore be robust to the detection of other, non-neutralizing
239 antibodies. Besides interfering factors that also occur in many other assays, such as
240 heterophilic antibodies or human anti-animal antibodies, immunogenic proteins of
241 closely related human coronaviruses can trigger cross-reactive antibodies in the host.
242 This has been known for many decades and led to the earlier categorization of
243 corona viruses into serogroups[31]. Cross-reactivity with serum samples from HCoV
244 patients has been shown for serological SARS-CoV-2 IgA and IgG antibody assays[4].
245 Therefore, in order to make a valid serological diagnosis of SARS-CoV-2 neutralizing
246 antibodies, it is essential to exclude cross-reactivity by a second confirmatory test.
247 This is even more important when, as in some commercial immunological test
248 systems, the SARS-CoV-2 nuclear protein or parts thereof are used as an antigen.
249 Unlike antibodies against the spike protein, antibodies against the nucleoprotein do

Journal Pre-proof

Journal Pre-proof

250 not have a neutralizing effect on SARS-CoV-2 because the nuclear protein is located
251 inside the virus and is therefore not directly accessible.

252 Widely accepted confirmatory tests, such as the virus neutralization test
253 recommended by the WHO during the SARS outbreak[32], are labour intensive,
254 resulting in slow sample throughput in diagnostic laboratories. The establishment of
255 highly specific primary screening assays that avoid false positive results and thus the
256 need for further confirmation is therefore an important objective. Surrogate
257 neutralization assays using pseudotyped virus particles that bear the Spike protein of
258 the SARS-CoV-2 virus do not require work inside high containment laboratories and
259 therefore might offer an alternative testing option in the near future[20,33].

260 Another challenge for the serological detection of SARS-CoV-2 immunity is the
261 possibility of a low antibody response in mildly infected or even asymptomatic
262 COVID-19 cases. Most severe SARS-CoV-2 infections lead to a robust immune
263 response[10], but on the other hand, PCR-diagnosed mild or asymptomatic infections
264 can cause variable humoral immune responses that might not be detected by
265 serological tests[20,34] or even fall below the detection limit in several patients within a
266 few weeks (Wölfel, unpublished data).

267 Cross-reactivity and cross-protectivity may be two sides of the same coin in COVID-
268 19, too. SARS-CoV-2 is closely related to HCoV-OC43 (another betacoronaviruses),
269 the most prevalent seasonal coronavirus detected among patients under the age of
270 five[16]. It has been hypothesized before that such a pre-existing cross-immunity may
271 confer protection and/or attenuate the severity of COVID-19[35]. Pre-existing cross-
272 protective immunity in individuals previously exposed to antigenically related
273 pathogens have already been demonstrated for pandemic influenza A H1N1 in
274 2009[36]. Polyclonal antibodies against SARS-CoV spike protein significantly inhibit the

Journal Pre-proof

Journal Pre-proof

275 entry of SARS-CoV-2 into the cell in mice[37], suggesting the possibility of an
276 mechanism analogous to influenza. Finally, it should be mentioned that relatively
277 nonspecific antibodies, such as might be produced by certain vaccination strategies,
278 are suspected of being able to enhance a pathological immune response[22,38].
279 However, first studies on vaccine antigens based on the RBD subunit of the S protein
280 did not show any evidence of such an antibody dependent enhancement[39].

281 **How do the available assay technologies differ in their conclusiveness?**

282 A growing number of in vitro diagnostic companies are developing SARS-CoV-2-
283 specific antibody tests (see https://www.finddx.org/covid-19/pipeline/). In addition to
284 the differences and problems with test performance described above, the different
285 assay techniques also differ in the conclusions that can be drawn from the results.
286 Table 1 gives an overview of assay techniques used in COVID-19 serology. Different
287 antigens (RBD, N, S1) have already been evaluated in various proprietary and
288 commercial ELISA methods[4]. Antigen selection is one of the crucial aspects of assay
289 development, that determines specificity, availability and scalability for mass
290 production. Recombinant proteins are produced either by prokaryotic or eukaryotic
291 expression systems[40]. Prokaryotic systems achieve higher production rates, but the
292 spectrum of suitable antigens is limited due to the lack of posttranslational
293 modification and may also influence their diagnostic performance[41]. Antigen
294 extraction from complete virus lysate is technically less complex, but requires the
295 availability of ultracentrifugation and a BSL3 containment. Raw lysates are of
296 particular interest in the early stages of outbreaks when purified proteins are not yet
297 available. After separation of the protein fractions, virus lysates for Western blotting
298 are used as a viable option for the validation of immunoassays and are also suitable
299 as confirmatory tests. Due to the high safety requirements these approaches for

Journal Pre-proof

Journal Pre-proof

300 antigen collection and diagnostic application are reserved for specialized
301 laboratories[42].

302 The general issue of low PPV demands either robust sensitivities above 99.99% or a
303 2-tier diagnostic process, i.e. positive screening tests have to be confirmed e.g. by
304 Western blot which is a serological standard for many decades.

305 Neutralization assays are the virological reference method for confirmation of
306 neutralizing antibodies. Plaque reduction neutralization tests and also more rapid
307 microneutralization tests have been described for SARS-Cov-2 antibody testing[14,42]
308 As all those techniques rely on usage of whole-virus preparations, they are limited to
309 biosafety level (BSL) 3 laboratories. Recently, an alternative assay that can be
310 performed under BSL 2 conditions was reported, employing a pseudovirus-based
311 assay to detect neutralizing antibodies against SARS-CoV-2[20]. The selection of
312 immunoglobulin isotypes is another feature that influences the informative value of an
313 assay. The direct comparison is still limited as at present only a few studies have
314 examined all three isotypes (i.e. IgG, IgA and IgM) in parallel[15,43]. IgA is supposed to
315 have a higher sensitivity compared to IgG Ab, while IgG is superior in specificity[4].
316 This observation mirrors the physiological importance of IgA as a polyreactive
317 antibody. Although polyreactivity is primarily considered a risk for autoimmune
318 diseases, it also offers superior defensive capabilities in the detection, neutralization
319 and elimination of pathogens[44].

320 **How to ensure the quality of available assays?**

321 As of early April 2020, 101 SARS-CoV-2 specific antibody tests, most of them rapid
322 point of care systems, have been CE-marked under EU Directive 98/79/EC
323 highlighting the currently still increasing diversity on the market[45,46]. The globally

Journal Pre-proof

Journal Pre-proof
324 acting non-governmental organization FIND provides an overview of current market
325 readiness of different test (see https://www.finddx.org/covid-19/pipeline/).

326 To ensure a high quality of diagnostic performance, laboratories have to adhere to
327 certain requirements comprising e.g. conduction of verification studies of
328 commercially available tests, use of internal quality controls and participation in
329 external quality assessment schemes (EQA). The rapid spread of COVID-19 and the
330 associated pandemic health crisis have put an intense time pressure on test
331 development by manufacturers and approval by governments and national
332 regulators. These circumstances justified the rapid declaration of kits by emergency
333 use authorization (EUA) systems[47,48]. As a consequence, laboratories might now be
334 forced to perform clinical validation studies to assure the quality of EUA kits. Thus,
335 anti-SARS-CoV-2 cross-reactivity with other types of coronaviruses[3] as a cause of
336 false-positive test results as well as the influence of other interfering factors such as
337 rheumatoid factors[38] must be clarified by the respective service provider. Furthermore,
338 the dynamic of the immune responses needs to be studied in detail to determine the
339 optimal time of diagnostics as false-negative test results might be attributed to inter-
340 individual differences in the immune response. To date, it has not been sufficiently
341 proven what influence the severity of the disease (asymptomatic, mild, severe) has
342 on the extent and course of detectable antibody responses[49]. The determination and
343 ideally standardization of cut-offs is one of the essential quality criteria that will affect
344 the intended use of COVID-19 serology. This is emphasized by WHO
345 recommending, as of 24th April 2020 scientific report, to restrict the use of SARS-
346 CoV-2 antibody testing to research settings until its diagnostic reliability is proven by
347 peer-reviewed large-scale studies and EQA schemes[40]. With the former currently
348 being conducted by WHO and FIND[50], reference material and EQA schemes are

Journal Pre-proof

349 currently only available for molecular-based SARS-CoV-2 testing[51-54]. The need is
350 further highlighted by former SARS-CoV EQA schemes that revealed a poor
351 sensitivity of 53% of EIA-based tests[55]. To meet this urgent demand, UK-NEQAS and
352 the Reference Institute for Bioanalytics (RfB) announced an upcoming EQA
353 scheme[57,58]. The RfB recently conducted the first pilot-scheme. Here, 8 serum
354 samples were provided for EIA-based testing of IgG, IgA and IgM. Preliminary results
355 of testing for IgG and IgA revealed a moderate concordance of assays with 66% and
356 75% agreement for IgG and IgA results between the laboratories, respectively.
357 Results submitted for IgM diverged substantially with only 25% of laboratories
358 reporting correct results for all samples provided (Haselmann et al. 2020 manuscript
359 in preparation). Notably, none of the participants correctly analyzed all samples.
360 Hence, further schemes providing serial dilutions of samples to stress analytical test
361 performance are mandatory.

362 Accuracy and reproducibility of the test formats is particularly important in the so
363 called "grey zone" in which immunity may not have developed completely. Facing a
364 high number of rapid lateral-flow tests with questionable quality flooding the
365 diagnostic market, certification by EQA schemes is one approach to select assays
366 with poor quality. In contrast, analytical and clinical validation of new test formats
367 require comprehensive testing in cohorts mirroring the natural prevalence of diverse
368 antibodies after a season of respiratory diseases and the indication of positive
369 predictive values under defined situations of varying prevalence.

370 With regard to the quality assurance of SARS-CoV-2 antibody tests, there is an
371 urgent need for suitable reference material, for large-scale validation studies
372 involving various available test systems, and for international proficiency testing
373 initiatives.

Journal Pre-proof

Journal Pre-proof

374 **Why should baseline samples be collected from still asymptomatic or healthy**
375 **individuals?**

376 There are different definitions of “baseline” samples and baseline studies. A blood
377 draw to obtain a baseline serum sample is recommended for contacts of infected
378 persons as early as possible within the incubation period of contact[59,60]. For patients
379 paired samples are necessary for confirmation with the initial (baseline) sample
380 collected in the first week of illness and the second ideally collected 2-4 weeks later
381 (the optimal timing for convalescent sample needs to be established)[59].
382 In a representative baseline study, a demographically representative cohort is
383 repeatedly tested to determine the rate of spread of the virus. This can be done by
384 serological analysis on blood donors, by studies in particularly affected places
385 ("hotspots") or nationwide in a carefully controlled population-representative study.
386 Baseline samples from non-infected healthy individuals are particularly important for
387 future validation purposes. Such stored serum samples can be used for future usage
388 as it can support diagnostics once validated serology tests are available[59,60].

Journal Pre-proof

389 The proactive storage of baseline samples, i.e. serum from individuals who were
390 CoViD-19-naïve at the time of blood collection, could speed up diagnostics as
391 seroconversion can be detected by parallel analysis of post-exposure samples
392 together with those initially collected. The absence of preformed cross-reacting
393 factors in baseline samples reduces the probability of unspecific positive results in
394 the follow-up sample in case of a suspected infection. Especially for the large number
395 of studies initiated at high speed for the prevention of COVID-19 there is an urgent
396 need to collect baseline samples. While accurate serological tests are still under
397 development, study participants are urgent to collect blood from study participants
398 awaiting such tests in the near future. These tests could become crucial to obtain

Journal Pre-proof

399 fully interpretable and unbiased results from these studies. For example, it has
400 recently been proposed to collect samples and data in advance to test the hypothesis
401 that resilience of the elderly during a pandemic can be improved by countering
402 chronic inflammation (inflammaging) and cellular senescence[61].

403 While this procedure is straightforward within studies, some countries may need
404 special regulations for implementation in the field of health care. At present it is
405 conceivable that biobanks are established with noble intentions but may then be
406 opened for purposes for which prior consent of the patient would have been required.
407 Similarly, this problem could also affect stored sera from employees. At this point, the
408 officials should verify the legitimacy of a proactive blood collection.

Journal Pre-proof

Journal Pre-proof

409 **Can laboratories estimate the medium-term demand for SARS-CoV-2 antibody**
410 **tests?**

411 Following the introduction of PCR methods, it soon became apparent that the
412 demand for test kits far exceeded their availability. A major difference between
413 molecular and serological diagnostics is that the latter can be performed in almost all
414 diagnostic laboratories; usually equipment is readily available. Personal
415 communication with the IVD industry currently estimates a demand only for a single
416 country like Germany between 2,000,000 and 5,000,000 tests per month. The
417 needed capacities may double as it can be assumed that most of the tested persons
418 have to be re-tested within one month. The assumed increase is also triggered by the
419 examination of contacts of persons tested positive in a low prevalence setting.
420 Production capacities of "high double-digit millions" per month" have already been
421 announced by one manufacturer. It therefore remains to be seen whether the
422 forecasts for both demand and availability will be met.

423 Mathematical models can help to estimate the period of increased demand based on
424 the duration of the pandemic. The German Robert Koch Institute modulated a SEIR
425 model on the rate of successfully isolated patients and the seasonality of disease
426 progression[62]. Seasonality leads to fluctuations of the basic reproduction number R[63]
427 and thereby markedly determines the length or even the end of an epidemic. Risks
428 like uncertainty about the duration of the pandemic or failing post-market surveillance
429 may lead some manufacturers to withdraw from the market. However, this will not
430 prevent others from capitalizing on the current supply shortages by fake products[64].

431 **PERSPECTIVE AND CONCLUSION**

Journal Pre-proof

Journal Pre-proof

432 Given an appropriate assay design, the serological testing of confirmed COVID-19
433 convalescent individuals can be expected to be accurate in detecting an anti-SARS-
434 CoV-2 response (importantly, a false negative result due to imperfect sensitivity will
435 not endanger the convalescent patient). All other positive results are due to
436 asymptomatic, previously undetected COVID-19 cases or are caused by non-SARS-
437 CoV-2-related cross-reactivities or unspecific test interferences. In general, a
438 specificity below 99.99%, i.e. 1 false positive within 10.000 true positive tests, in a
439 low prevalence setting (< 1%) will generate a number of false positives inversely
440 related to the prevalence of the biomarkers tested. This may lead to a systematic
441 overestimation of the prevalence of immunity in the population as well as lower
442 estimates of virus mortality rate and pose a challenge for any subsequent clinical,
443 societal and economic decision-making.
444 Future studies therefore need to concentrate on three aspects: i) using test systems
445 with 100% SARS-CoV-2 patient antibody specificities, preferably capable to detect
446 antibodies blocking virus-cell interaction as candidates for protective immunity. While
447 there is some promise with the immune testing systems available, the current tests
448 have not shown the specificities needed to warrant the interpretation of positive
449 results in screening situations. ii) controlling the prevalence in the population groups
450 tested in a dynamic fashion. This may be accomplished by contact tracing in the case
451 of a positive virus finding, thus allowing to improve the prevalence in the social
452 surroundings of the individual tested positive (confirmed niche testing). iii)
453 Furthermore, overestimation of prevalence can be quickly corrected by avoiding
454 selection bias in the study cohort.

Journal Pre-proof

Journal Pre-proof

455 **KEY FACTS**

456 • Seroconversion when SARS-CoV-2 is detected by RT-PCR indicates a SARS-
457 CoV-2 specific humoral immune response.

458 • In screening situations, the number of false positive results is inversely correlated
459 to the prevalence of the disease for any test with specificity below 100%.

460 • The response characteristics in sub- and oligo-symptomatic clinical infections, a
461 significant proportion of SARS-CoV-2 infections, remains a key gap in the
462 literature.

463 • It is currently unknown whether the available serological assays can be used to
464 confirm immunity against SARS-CoV-2.

465 • Even though more than 100 different antibody tests are currently available, global
466 and territorial seroprevalence of CoViD-19 remains unknown.

467 **ACKNOWLEDGEMENT**

468 We gratefully acknowledge Tobias Kiehntopf for developing and providing the
469 software that was used to create Figure 2 and to calculate the prevalence dependent
470 predictive values shown therein. We thank Claudia Trier for excellent editorial
471 assistance.

Journal Pre-proof

Journal Pre-proof

472 **REFERENCES**

473 1. Meyer B, Drosten C, Müller MA. Serological assays for emerging
474 coronaviruses: Challenges and pitfalls. Virus Res. 2014;194:175–83. doi:
475 10.1016/j.virusres.2014.03.018

476 2. Jin Y, Wang M, Zuo Z, Fan C, Ye F, Cai Z, et al. Diagnostic value and
477 dynamic variance of serum antibody in coronavirus disease 2019. Int J Infect
478 Dis. 2020;94:49–52. doi: 10.1016/j.ijid.2020.03.065

479 3. Liu W, Liu L, Kou G, Zheng Y, Ding Y, Ni W, et al. Evaluation of Nucleocapsid
480 and Spike Protein-based ELISAs for detecting antibodies against SARS-CoV-
481 2. J Clin Microbiol. 2020; doi: 10.1128/jcm.00461-20 [Epub ahead of print]

482 4. Okba NMA, Müller MA, Li W, Wang C, GeurtsvanKessel CH, Corman VM,
483 Lamers MM, et al. Severe Acute Respiratory Syndrome Coronavirus 2-
484 Specific Antibody Responses in Coronavirus Disease 2019 Patients. Emerg
485 Infect Dis. 2020;26. doi: 10.3201/eid2607.200841

486 5. Tan W, Lu Y, Zhang J, Wang J, Dan Y, Tan Z, et al. Viral Kinetics and
487 Antibody Responses in Patients with COVID-19. Preprint at medRxiv. 2020;
488 doi: 10.1101/2020.03.24.20042382

489 6. To KK-W, Tsang OT-Y, Leung W-S, Tam AR, Wu T-C, Lung DC, et al.
490 Temporal profiles of viral load in posterior oropharyngeal saliva samples and
491 serum antibody responses during infection by SARS-CoV-2: an observational
492 cohort study. Lancet Infect Dis. 2020;20(5):565–74. doi: 10.1016/s1473-
493 3099(20)30196-1

Journal Pre-proof

Journal Pre-proof

494 7. Xiang F, Wang X, He X, Peng Z, Yang B, Zhang J, et al. Antibody Detection
495 and Dynamic Characteristics in Patients with COVID-19. Clin Infect Dis. 2020;
496 doi: 10.1093/cid/ciaa461 [Epub ahead of print]

497 8. Xiao AT, Gao C, Zhang S. Profile of specific antibodies to SARS-CoV-2: The
498 first report. J Infect. 2020; doi: 10.1016/j.jinf.2020.03.012 [Epub ahead of print]

499 9. Zeng Z, Chen L, Pan Y, Deng Q, Ye G, Li Y, et al. Re: Profile of specific
500 antibodies to SARS-CoV-2: The first report. J Infect. 2020; doi:
501 10.1016/j.jinf.2020.03.052 [Epub ahead of print]

502 10. Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et al. Antibody responses to
503 SARS-CoV-2 in patients of novel coronavirus disease 2019. Clin Infect Dis.
504 2020; doi: 10.1093/cid/ciaa344 [Epub ahead of print]

505 11. Petherick A. Developing antibody tests for SARS-CoV-2. Lancet.
506 2020;395:1101–2. doi: 10.1016/S0140-6736(20)30788-1

507 12. Kucirka L, Lauer S, Laeyendecker O, Boon D, Lessler J. Variation in False
508 Negative Rate of RT-PCR Based SARS-CoV-2 Tests by Time Since
509 Exposure. Preprint at medRxiv. 2020; doi: 10.1101/2020.04.07.20051474

510 13. Wikramaratna P, Paton RS, Ghafari M, Lourenco J. Estimating false-negative
511 detection rate of SARS-CoV-2 by RT-PCR. Preprint at medRxiv. 2020; doi:
512 10.1101/2020.04.05.20053355

513 14. Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, et al.
514 Virological assessment of hospitalized patients with COVID-2019. Nature.
515 2020; doi: 10.1038/s41586-020-2196-x [Epub ahead of print]

Journal Pre-proof

Journal Pre-proof

516 15. Guo L, Ren L, Yang S, Xiao M, Chang D, Yang F, et al. Profiling Early
517 Humoral Response to Diagnose Novel Coronavirus Disease (COVID-19). Clin
518 Infect Dis. 2020; doi: 10.1093/cid/ciaa310 [Epub ahead of print]

519 16. Zhou W, Wang W, Wang H, Lu R, Tan W. First infection by all four non-severe
520 acute respiratory syndrome human coronaviruses takes place during
521 childhood. BMC Infect Dis. 2013;13(1). doi: 10.1186/1471-2334-13-433

522 17. Yongchen Z, Shen H, Wang X, Shi X, Li Y, Yan J, et al. Different longitudinal
523 patterns of nucleic acid and serology testing results based on disease severity
524 of COVID-19 patients. Emerg Microbes Infect. 2020;9:833–6. doi:
525 10.1080/22221751.2020.1756699 [Epub ahead of print]

526 18. Roche Diagnostics. Elecsys Anti-SARS-CoV-2 - Instructions for Use.
527 2020 May 17 Available at: https://www.fda.gov/media/137605/download .

528 19. Shen C, Wang Z, Zhao F, Yang Y, Li J, Yuan J, et al. Treatment of 5 Critically
529 Ill Patients With COVID-19 With Convalescent Plasma. JAMA. 2020;323:1582.
530 doi: 10.1001/jama.2020.4783 [Epub ahead of print]

531 20. Wu F, Wang A, Liu M, Wang Q, Chen J, Xia S, et al. Neutralizing antibody
532 responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their
533 implications. Preprint at medRxiv. 2020; doi: 10.1101/2020.03.30.20047365

534 21. Ye M, Fu D, Ren Y, Wang F, Wang D, Zhang F, et al. Treatment with
535 convalescent plasma for COVID-19 patients in Wuhan, China. J Med Virol.
536 2020; doi: 10.1002/jmv.25882 [Epub ahead of print]

Journal Pre-proof

Journal Pre-proof

537 22. Iwasaki A, Yang Y. The potential danger of suboptimal antibody responses in
538 COVID-19. Nat Rev Immunol. 2020; doi: 10.1038/s41577-020-0321-6 [Epub
539 ahead of print]

540 23. Mandavilli A. Coronavirus Antibody Tests: Can You Trust the Results? New
541 York Times 2020 April 27 Available at:
542 https://www.nytimes.com/2020/04/24/health/coronavirus-antibody-tests.html

543 24. Whitman JD, Hiatt J, Mowery CT, Shy BR, Yu R, Yamamoto TN, et al. Test
544 performance evaluation of SARS-CoV-2 serological assays. Preprint at
545 medRxiv. 2020; doi: 10.1101/2020.04.25.20074856

546 25. Bendavid E, Mulaney B, Sood N, Shah S, Ling E, Bromley-Dulfano R, et al.
547 COVID-19 Antibody Seroprevalence in Santa Clara County, California.
548 Preprint at medRxiv. 2020; doi: 10.1101/2020.04.14.20062463

549 26. Gudbjartsson DF, Helgason A, Jonsson H, Magnusson OT, Melsted P,
550 Norddahl GL, et al. Spread of SARS-CoV-2 in the Icelandic Population. N Engl
551 J Med. 2020; doi: 10.1056/nejmoa2006100 [Epub ahead of print]

552 27. Ai T, Yang Z, Hou H, Zhan C, Chen C, Lv W, et al. Correlation of Chest CT
553 and RT-PCR Testing in Coronavirus Disease 2019 (COVID-19) in China: A
554 Report of 1014 Cases. Radiology. 2020;200642. doi:
555 10.1148/radiol.2020200642 [Epub ahead of print]

556 28. Kabesch M, Roth S, Brandstetter S, Häusler S, Juraschko E, Weigl M, et al.
557 Successful containment of COVID-19 outbreak in a large maternity and
558 perinatal center while continuing clinical service. Pediatr Allergy Immunol.
559 2020; doi: 10.1111/pai.13265 [Epub ahead of print]

Journal Pre-proof

Journal Pre-proof

560 29. Hussain S, Pan J, Chen Y, Yang Y, Xu J, Peng Y, et al. Identification of Novel
561 Subgenomic RNAs and Noncanonical Transcription Initiation Signals of
562 Severe Acute Respiratory Syndrome Coronavirus. J Virol. 2005;79:5288–95.
563 doi: 10.1128/jvi.79.9.5288-5295.2005

564 30. Zhang W, Du R-H, Li B, Zheng X-S, Yang X-L, Hu B, et al. Molecular and
565 serological investigation of 2019-nCoV infected patients: implication of multiple
566 shedding routes. Emerg Microbes Infect. 2020;9:386–9. doi:
567 10.1080/22221751.2020.1729071

568 31. McIntosh K, Kapikian AZ, Hardison KA, Hartley JW, Chanock RM. Antigenic
569 relationships among the coronaviruses of man and between human and
570 animal coronaviruses. J Immunol. 1969;102:1109–18.

571 32. World Health Organisation. WHO Guidelines for the Global Surveillance of
572 Severe Acute Respiratory Syndrome (SARS): Updated recommendations,
573 October 2004. Publication Server of WHO. 2004;Vol. 2013.

574 33. Grzelak L, Temmam S, Planchais C, Demeret C, Huon C, Guivel F, et al.
575 SARS-CoV-2 serological analysis of COVID-19 hospitalized patients, pauci-
576 symptomatic individuals and blood donors. Preprint at medRxiv. 2020; doi:
577 10.1101/2020.04.21.20068858

578 34. van der Heide V. Neutralizing antibody response in mild COVID-19. Nat Rev
579 Immunol. 2020; doi: 10.1038/s41577-020-0325-2 [Epub ahead of print]

580 35. Nickbakhsh S, Ho A, Marques DFP, McMenamin J, Gunson RN, Murcia PR.
581 Epidemiology of Seasonal Coronaviruses: Establishing the Context for the
582 Emergence of Coronavirus Disease 2019. J Infect Dis. 2020; doi:
583 10.1093/infdis/jiaa185 [Epub ahead of print]

Journal Pre-proof

Journal Pre-proof

584 36. Kerkhove MD Van, Hirve S, Koukounari A, Mounts AW. Estimating age-
585 specific cumulative incidence for the 2009 influenza pandemic: a meta-
586 analysis of A(H1N1)pdm09 serological studies from 19 countries. Influenza
587 Other Respi Viruses. 2013;7:872–86. doi: 10.1111/irv.12074 [Epub ahead of
588 print]

589 37. Walls AC, Park Y-J, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure,
590 Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. Cell.
591 2020;181:281--292.e6. doi: 10.1016/j.cell.2020.02.058 [Epub ahead of print]

592 38. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ.
593 COVID-19: consider cytokine storm syndromes and immunosuppression.
594 Lancet. 2020;395:1033–4. doi: 10.1016/s0140-6736(20)30628-0 [Epub ahead
595 of print]

596 39. Quinlan BD, Mou H, Zhang L, Guo Y, He W, Ojha A, et al. The SARS-CoV-2
597 Receptor-Binding Domain Elicits a Potent Neutralizing Response Without
598 Antibody-Dependent Enhancement. SSRN Electron J. 2020; doi:
599 10.2139/ssrn.3575134

600 40. Stadlbauer D, Amanat F, Chromikova V, Jiang K, Strohmeier S, Arunkumar
601 GA, et al. SARS-CoV-2 Seroconversion in Humans: A Detailed Protocol for a
602 Serological Assay, Antigen Production, and Test Setup. Curr Protoc Microbiol.
603 2020;57. doi: 10.1002/cpmc.100 [Epub ahead of print]

604 41. Zhang P, Gao Q, Wang T, Ke Y, Mo F, Jia R, et al. Evaluation of recombinant
605 nucleocapsid and spike proteins for serological diagnosis of novel coronavirus
606 disease 2019 (COVID-19). Preprint at medRxiv. 2020; doi:
607 10.1101/2020.03.17.20036954

Journal Pre-proof

Journal Pre-proof

608 42. Haveri A, Smura T, Kuivanen S, Österlund P, Hepojoki J, Ikonen N, et al.
609 Serological and molecular findings during SARS-CoV-2 infection: the first case
610 study in Finland, January to February 2020. Eurosurveillance. 2020;25(11).
611 doi: 10.2807/1560-7917.es.2020.25.11.2000266 [Epub ahead of print]

612 43. Zhang Z, Xiao T, Wang Y, Yuan J, Ye H, Wei L, et al. Early viral clearance and
613 antibody kinetics of COVID-19 among asymptomatic carriers. Preprint at
614 medRxriv. 2020; doi: 10.1101/2020.04.28.20083139

615 44. Dimitrov JD, Planchais C, Roumenina LT, Vassilev TL, Kaveri S V, Lacroix-
616 Desmazes S. Antibody Polyreactivity in Health and Disease: Statu Variabilis. J
617 Immunol. 2013;191:993–9. doi: 10.4049/jimmunol.1300880 [Epub ahead of
618 print]

619 45. Commission of the European Union. Communication from the Commission -
620 Guidelines on COVID-19 in vitro diagnostic tests and their performance. Off J
621 Eur Union 2020/C 122 I/01.

622 46. Vashist SK. In Vitro Diagnostic Assays for COVID-19: Recent Advances and
623 Emerging Trends. Diagnostics. 2020;10:202. doi:
624 10.3390/diagnostics10040202 [Epub ahead of print]

625 47. Food and Drug Administration. Policy for Coronavirus Disease-2019 Tests
626 During the Public Health Emergency (Revised). 2020 May 11 Available at:
627 https://www.fda.gov/media/135659/download

628 48. Hong KH, Lee SW, Kim TS, Huh HJ, Lee J, Kim SY, et al. Guidelines for
629 Laboratory Diagnosis of Coronavirus Disease 2019 (COVID-19) in Korea. Ann
630 Lab Med. 2020;40:351–60. doi: 10.3343/alm.2020.40.5.351 [Epub ahead of
631 print]

Journal Pre-proof

Journal Pre-proof

632 49. Huang AT, Garcia-Carreras B, Hitchings MDT, Yang B, Katzelnick L, Rattigan
633 SM, et al. A systematic review of antibody mediated immunity to
634 coronaviruses: antibody kinetics, correlates of protection, and association of
635 antibody responses with severity of disease. Ann Lab Med. 2020; doi:
636 10.1101/2020.04.14.20065771 [Epub ahead of print]

637 50. Foundation for Innovative New Diagnostics. FIND evaluation update: SARS-
638 CoV-2 immunoassays. 2020 April 27 Available at: https//www.finddx.org/covid-
639 19/sarscov2-eval-immuno/

640 51. AccuPlex. SARS-CoV-2 Reference Material Kit. 2020 April 28
641 https//www.seracare.com/AccuPlex-SARSCoV2-Reference-Material-Kit-0505-
642 0126/

643 52. Zeichhardt H, Kammel M. Gesellschaft zur Förderung der Qualitätssicherung
644 in medizinischen Laboratorien e.V. Novel coronavirus SARS-CoV-2 (2019-
645 nCoV) includeding the next INSTAND EQA scheme. 28 April 2020 Available at
646 https//www.instand-
647 ev.de/fileadmin/uploads/user_upload/Dokumente/Virologie/20200214_INSTAN
648 D_announcement_EQAS_novel_coronavirus_SARS-CoV-2.pdf

649 53. Laboratory of the Government Chemist Group. COV - SARS-CoV-2 molecular
650 proficiency test. 2020 April 28 Available at:
651 https//us.lgcstandards.com/US/en/COV-SARS-CoV-2-molecular/p/PT-CL-MI-
652 COV

653 54. Randox. Quality Control for Molecular Diagnostics. Coronavirus Outbreak
654 Preparedness EQA Pilot Study. 2020 April 28 Available at:
655 https//www.randox.com/sars-cov-2-eqa-scheme/

Journal Pre-proof

Journal Pre-proof

656 55. College of American Pathologists. SARS-CoV-2 Molecular Proficiency Testing
657 Program. 2020 April 28 Available at: https//www.cap.org/laboratory-
658 improvement/proficiency-testing/sars-cov-2-molecular

659 56. Niedrig M, Leitmeyer K, Lim W, Peiris M, Mackenzie JS, Zambon M. First
660 external quality assurance of antibody diagnostic for SARS-new coronavirus. J
661 Clin Virol. 2005;34:22–5. doi: 10.1016/j.jcv.2005.01.004

662 57. United Kingdom National External Quality Assessment Service. New COVID-
663 19 UK NEQAS Pilot Scheme Developments. 2020 May 16 Avalable at:
664 https//ukneqas.org.uk/news/new-covid-19-uk-neqas-pilot-scheme-
665 developments/

666 58. Referenzinstitut für Bioanalytik. Application survey SARS-CoV-2 Immunology.
667 2020 May 2020 Available at:
668 https//rfb.bio/pdf/2020/Ankuendigung_Anmeldung_CoV_2020_de_en.pdf

669 59. World Health Organization. Laboratory testing for 2019 novel coronavirus
670 (2019-nCoV) in suspected human cases, Interim guidance, 19 March 2020.
671 Publication Server of WHO.

672 60. European Centre for Disease Prevention and Control. Laboratory support for
673 COVID-19 in the EU/EEA. 2020 April 27 Available at:
674 https//www.ecdc.europa.eu/en/novel-coronavirus/laboratory-support

675 61. Fuellen G, Liesenfeld O, Kowald A, Barrantes I, Bastian M, et al. The
676 preventive strategy for pandemics in the elderly is to collect in advance
677 samples & data to counteract chronic inflammation (inflammaging). Ageing
678 Res Rev. 2020 doi: 10.1016/j.cytogfr.2020.04.005 [Epub ahead of print]

Journal Pre-proof

Journal Pre-proof

679 62. an der Heiden M, Buchholz U. Modellierung von Beispielszenarien der SARS-
680 CoV-2-Epidemie 2020 in Deutschland. Publication Server of Robert Koch
681 Institute. 2020; doi: 10.25646/6571.2

682 63. Kissler SM, Tedijanto C, Goldstein E, Grad YH, Lipsitch M. Projecting the
683 transmission dynamics of SARS-CoV-2 through the postpandemic period.
684 Science. 2020; eabb5793. doi: 10.1126/science.abb5793 [Epub ahead of print]

685 64. World Health Organization. Medical Product Alert N°3/2020. Falsified medical
686 products, including in vitro diagnostics, that claim to prevent, detect, treat or
687 cure COVID-19. 2020 March 31 Available at: https://www.who.int/news-
688 room/detail/31-03-2020-medical-product-alert-n-3-2020

689

690

Journal Pre-proof

Journal Pre-proof

691 **LEGENDS TO FIGURE**

692 **Figure 1**

693 Positive predicted values for 21 commercial SARS-CoV-2 immunoassays and 1 lab-
694 developed assay detecting IgM and IgG antibodies (total of 14 test systems) in
695 patient sera and controls. Data were extracted from Whitman et al[24]. and plotted
696 against various prevalence settings (0.08% to 25.6%).
697 Assays evaluated: M: Inhouse; K: Epitope Diagnostics IgG; I2: VivaChek IgG; H2:
698 UCP IgG; G2: Sure IgG; F2: Premier IgG; E2: Innovita IgG; D2: DeepBlue IgG; C2:
699 Decombio IgG; B2: Bioperfectus IgG; A2: Biomedomics IgG; L: Wondito IgG/IgM; K1:
700 Epitope Diagnostics IgM; I1: VivaChek IgM; H1: UCP IgM; G1: Sure IgM; F1: Premier
701 IgM; E1: Innovita IgM; D1: DeepBlue IgM; C1: Decombio IgM; B1: Bioperfectus IgM;
702 A1: BioMedomics IgM.

703 **Figure 2**

704 Examples of PPV (A) and NPV (B) values (y-axis) as a function of prevalence (x-
705 axis). Gray lines illustrate a theoretically assumed range of test
706 sensitivities/specificities from 80/80% to 99.9/99.9%, as indicated, respectively.
707 Two commercially available SARS-CoV-2 IgG tests are shown with (A) specificities of
708 90.63% (blue) and 98.5% (red), and (B) sensitivities of 88.66% (blue) and 80% (red)
709 respectively. PPV for a population-based prevalence of 0.24% for COVID-19
710 (Regensburg, Bavaria) and 0.9%[26] are illustrated in the insert of plot (A). As obvious
711 in (B), even though assay sensitivity is only 80% due to its higher specificity the red
712 line is located above the grey line that indicates prevalence dependent NPV for
713 sensitivities/specificities of 80%, respectively.

Journal Pre-proof

Journal Pre-proof

714 **TAB 1.** Synopsis of available SARS-CoV-2 serological techniques.

715 EIA: Enzyme-Immunoassay; IFT: Immunofluorescence Test; DB/WB: Dot blot/
716 Western blot; LFA: Immunochromatographic lateral flow assays; VNT: Virus
717 Neutralization Assay.

Technique	Rationale for usage	Advantages	Disadvantages
EIA	monitoring of seroconversion; contact tracing; seroprevalence studies	high throughput; availability, easy to perform	lack of knowledge on utilization and quality; inability to confirm antibodies (neutralization) functionality
IFT	monitoring of seroconversion; seroprevalence studies	no analyzer (but IF microscope) needed	low throughput; experience required; discrimination of other CoV antibodies; time-consuming
DB/WB	confirmatory; proof of specificity/ cross-reactivity; research use	discrimination of other coronavirus antibodies	not commonly available; experience required (WB)
VNT	confirmatory; proof of specificity/ cross-reactivity; virological reference method	functional information	BSL3-Lab necessary
LFA	lack of other resources	independent from lab equipment	questionable sensitivity and specificity

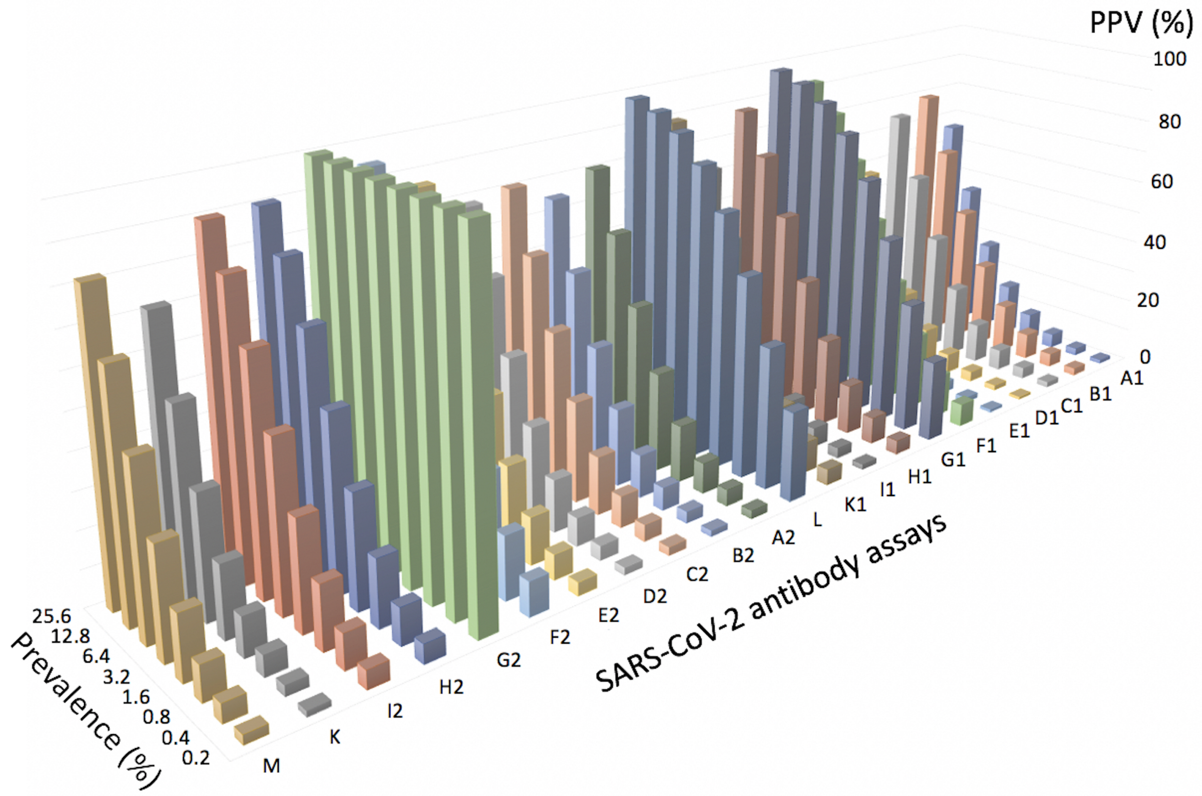
718

Journal Pre-proof

Journal Pre-proof

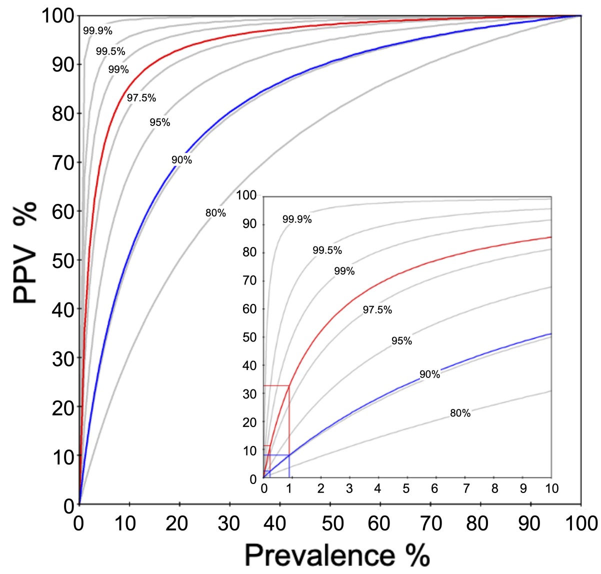
719

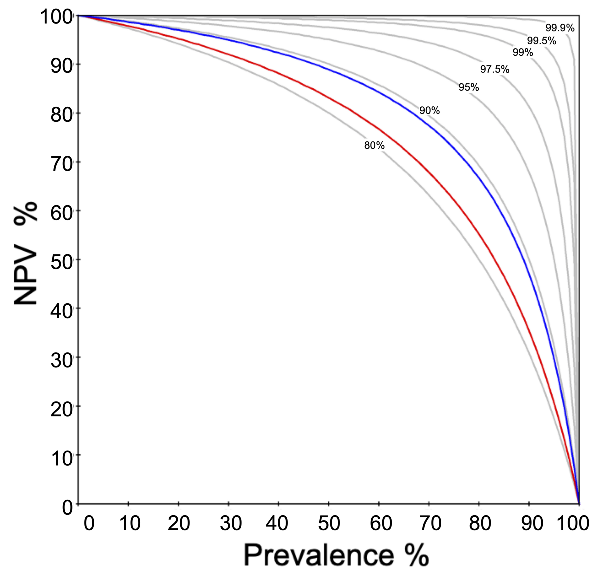
Journal Pre-proof



Journal

Journal Pre-proof





Journal Pre-proof