JCM Accepted Manuscript Posted Online 12 August 2020
J. Clin. Microbiol. doi:10.1128/JCM.01695-20
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1 **Challenges and Controversies Related to Testing for COVID-19**

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25 **Abstract**

26 The coronavirus disease (COVID)-19 pandemic has placed the clinical laboratory and testing for SARS-
27 CoV-2 front-and-center in the worldwide discussion of how to end the outbreak. Clinical laboratories
28 have responded by developing, validating and implementing a variety of molecular and serologic assays
29 to test for SARS-CoV-2 infection. This has played an essential role in identifying cases, informing isolation
30 decisions and helping to curb the spread of disease. However, as the demand for COVID-19 testing has
31 increased, laboratory professionals have faced a growing list of challenges, uncertainties and, in some
32 situations, controversy, as they have attempted to balance the need for increasing test capacity with
33 maintaining a high-quality laboratory operation. The emergence of this new viral pathogen has raised
34 unique diagnostic questions for which there have not always been straightforward answers. In this
35 commentary, the author addresses several areas of current debate, including 1) the role of molecular
36 assays in defining the duration of isolation/quarantine, 2) whether the PCR cycle threshold value should
37 be included on patient reports, 3) if specimen pooling and testing by research staff represent acceptable
38 solutions to expand screening, and 4) whether testing a large percentage of the population is feasible
39 and represents a viable strategy to end the pandemic.

40

41 **Introduction**

42 The coronavirus disease (COVID)-19 pandemic, caused by severe acute respiratory syndrome
43 coronavirus (SARS-CoV)-2, has been confirmed in nearly 20 million cases and caused over 730,000
44 deaths worldwide (https://coronavirus.jhu.edu). As the virus has spread across the globe, laboratory
45 testing has played an important role in diagnosing those with disease, as well as identifying individuals
46 who are asymptomatic yet have the potential to serve as a source of viral transmission. Molecular tests,
47 such as real-time PCR, have been the most common laboratory tool used to detect cases of COVID-19. In
48 fact, SARS-CoV-2 molecular assays have become an integral component in a multi-pronged strategy

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49 aimed at reducing transmission of the virus. This strategy has consisted of 1) case identification (i.e.,
50 through testing), 2) quarantine or isolation of exposed/infected individuals, and 3) contact tracing.
51 Despite the broad application of this strategy, cases of COVID-19 have continued to surge, especially in
52 the United States where the number of confirmed infections has surpassed 5 million (~25% of the global
53 case count).

54 The COVID-19 pandemic is an unprecedented healthcare crisis, which has required clinical and
55 laboratory professionals to rapidly adapt to new information, innovate and, in some situations,
56 implement practices that would not be considered under normal circumstances. The emergence of
57 SARS-CoV-2 has forced clinical laboratories and test manufacturers to develop novel diagnostic assays in
58 a timeframe that previously would not have been considered feasible. As testing options have become
59 available, healthcare professionals and diagnostics experts have had to learn how to best apply these
60 tools to diagnose and manage patients with COVID-19 and slow the spread of disease. Some of these
61 lessons have shown routine testing approaches to be effective, while others have taught us that a new
62 pathogen, such as SARS-CoV-2, may require a new diagnostic playbook.

63 As SARS-CoV-2 has spread globally, it has required laboratory professionals to venture into
64 uncharted territory. Not only is testing being used to diagnose patients with symptomatic disease, it is
65 being incorporated into local, state and federal strategies to reopen the economy. Businesses, schools
66 and even athletic teams are considering how laboratory testing can be used to demonstrate that
67 asymptomatic employees, customers, students and athletes are safe to return to normal activities.
68 Some hospitals and clinics are utilizing SARS-CoV-2 testing prior to certain procedures and surgeries to
69 reduce nosocomial transmission and prevent poor outcomes in high-risk patients. This trend towards
70 large-scale screening of asymptomatic individuals has placed an incredible burden on the global testing
71 infrastructure and has created challenges with regard to how testing should be utilized and results
72 interpreted. Therefore, it is not surprising that a number of diagnostic uncertainties and controversies

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73 have arisen. In this commentary, I will address several topics that have been the focus of continued
74 discussion and debate, including 1) the clinical sensitivity of molecular assays and their role in defining
75 the duration of quarantine/isolation, 2) whether the PCR cycle threshold value should be included on
76 patient reports, 3) the potential for specimen pooling and testing by research staff to expand testing
77 capacity, and 4) whether testing a large percentage of the population represents a viable strategy to end
78 the pandemic.
79
80 ***The clinical sensitivity of PCR and its use as a test-of-cure***
81 Molecular tests, such as real-time PCR, have become a cornerstone in the diagnosis of infectious
82 diseases and have been the most common laboratory method utilized during the COVID-19 pandemic.
83 The inherent sensitivity of real-time PCR allows for detection of minute amounts (e.g., <100 copies/mL)
84 of target nucleic acid in clinical samples. Despite this, one of the earliest challenges arising during the
85 COVID-19 pandemic was the concern that cases were going undetected by real-time PCR (1 – 3). Several
86 studies reported the clinical sensitivity of SARS-CoV-2 real-time PCR assays performed on upper
87 respiratory swab samples to be in the range of 60-70% (4, 5). However, as experience was gained with
88 testing and additional data published, it was recognized that the likelihood of detecting SARS-CoV-2 RNA
89 is highly dependent on the timing of sample collection, the type of specimen that is obtained and the
90 quality of the sample. SARS-CoV-2 is present at the highest amounts in the upper respiratory tract (URT)
91 (i.e., the nasopharynx) during the first several days following symptom onset (typically 5-7 days
92 following exposure), and subsequently declines in the URT over the course of the following week (6).
93 During the later stages of disease (e.g., >7 days post onset of symptoms), lower respiratory tract (LRT)
94 samples, such as sputum, bronchoalveolar lavage (BAL) fluid or tracheal secretions may yield higher
95 rates of detection (4). Due to these nuances, it has been challenging for laboratory professionals to truly
96 define the clinical sensitivity of SARS-CoV-2 real-time PCR and has required that negative results be

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97 interpreted in the context of the timing of sample collection (early post-onset *versus* late post-onset),
98 the type of specimen tested (e.g., NP swab *versus* throat swab) and the performance characteristics of
99 the assay.

100 Despite the observation that some COVID-19 patients initially test negative by real-time PCR (1,
101 2), a dichotomous observation has been the persistent detection of SARS-CoV-2 RNA in other patients
102 (7). A number of published reports have demonstrated that SARS-CoV-2 real-time PCR assays may be
103 positive for weeks, even following the resolution of clinical symptoms (7, 8). The author is aware of a
104 case where a patient with an underlying health condition tested positive by PCR for over 100 days
105 (unpublished data). Many healthcare institutions have followed a test-based strategy – initially
106 recommended by the U.S. Centers for Disease Control and Prevention (CDC) – in which two negative
107 PCR results (obtained on serial samples collected at least 24 hours apart) were required prior to
108 releasing a patient from isolation. This approach, whereby qualitative molecular assays have been used
109 to assess whether an individual is infectious has led to prolonged isolation, loss of work, extended use of
110 personal protective equipment for hospitalized patients and psychological distress for patients and their
111 family members.

112 Laboratory professionals have recognized for years that qualitative molecular assays for
113 infectious diseases can remain positive following the resolution of disease, and therefore, these
114 methods are not typically recommended as "tests-of-cure" (9, 10). Due to this limitation, the U.S. CDC
115 no longer recommends the use of a SARS-CoV-2 test-based strategy to determine when to discontinue
116 transmission-based precautions, instead relying on a symptom-based strategy in the majority of
117 situations (https://www.cdc.gov/coronavirus/2019-ncov/hcp/duration-isolation.html).
118
119 ***Author's opinion:*** Molecular methods, including real-time PCR, should not be used following an initial
120 diagnosis of COVID-19 to determine whether an individual continues to shed infectious SARS-CoV-2.

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121 Repeat molecular testing may be indicated in patients who recover and subsequently develop new,
122 COVID-19 related symptoms.
123
124 ***Providing the PCR cycle threshold value in the patient report***
125 Due to the possibility that SARS-CoV-2 molecular assays can remain persistently reactive in patients who
126 have recovered from COVID-19 or remain asymptomatic, there has been interest in whether semi-
127 quantitative or quantitative data can assist in result interpretation. For many commercial and
128 laboratory-developed real-time PCR assays, the cycle threshold (C_t) value associated with a PCR result is
129 available to the laboratory staff. The C_t value is inversely proportional to the amount of target nucleic
130 acid and can be used as a relative indicator of the concentration of a pathogen in a clinical specimen. For
131 example, a positive PCR result with an associated C_t value of 15 would indicate a very high concentration
132 of the target nucleic acid in a sample, whereas a C_t of 35 may suggest that the target is present but near
133 the assay's limit of detection. Importantly, the C_t value is non-normalized, and therefore, cannot be
134 considered a quantitative result. This is because the C_t value is dependent on a number of variables,
135 including the assay's gene target, the extraction platform, PCR amplification chemistry and even the
136 quality of specimen collection.
137 Despite these limitations, several studies have assessed whether a correlation can be made
138 between the PCR C_t value and the presence of replication-competent virus, by using viral culture as a
139 surrogate for a patient's infectious status. Bullard *et al* (11) compared viral culture and C_t values for 90
140 respiratory samples that were positive by PCR and demonstrated that a C_t >24 showed a strong
141 correlation with reduced recovery of SARS-CoV-2 in cell culture. Similarly, La Scola *et al* inoculated 183
142 PCR positive respiratory samples in a Vero E6 cell line (12). This group demonstrated a similar reduction
143 in culture positivity as the C_t value increased; however, in this study, a C_t >34 was proposed as a
144 threshold to estimate that an individual is no longer shedding infectious virus. Unfortunately, PCR C_t

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145 values may vary significantly between assays, even those using the same gene target (13, 14). During the
146 COVID-19 pandemic, it has become common for clinical laboratories to perform multiple real-time PCR
147 assays to detect SARS-CoV-2. Therefore, including the C_t value on all positive results may be confusing
148 and misleading to ordering providers. Since the C_t value is not normalized against a human gene internal
149 control, it is possible that a high C_t value (e.g., >30) could be due to an inadequate sample collection
150 rather than a low level of target nucleic acid or 'non-infectious' virus. Additional data are needed before
151 a definitive viral load threshold correlating with infectivity can be established. Finally, we should be
152 cautious to equate viral culture negativity with an individual's inability to serve as a source of viral
153 transmission. Due to inferior sensitivity, viral culture has been replaced by molecular assays for the
154 diagnosis of a number of respiratory viral infections.
155
156 ***Author's opinion***: The PCR C_t value for qualitative, SARS-CoV-2 real-time PCR assays should not be
157 routinely reported. On a case-by-case basis, the C_t value may be provided (i.e., verbally) to the ordering
158 physician, upon request. This approach allows for clarification of the assay used for testing and a
159 discussion of the limitations associated with using the C_t value while interpreting the result.
160
161 ***Specimen pooling as an approach to increase testing capacity***
162 One of the most challenging aspects of the COVID-19 pandemic for clinical laboratories has been
163 ongoing supply chain shortages and disruptions. Laboratories have not only struggled with maintaining
164 an adequate supply of test reagents, but also other essential supplies, including consumables (e.g., pipet
165 tips, 96-well plates), swabs and viral transport media. As the demand for testing has increased alongside
166 a global shortage of necessary supplies, laboratory professionals have been forced to identify ways to
167 "do more with less." One potential solution receiving a significant amount of attention is specimen
168 pooling, whereby aliquots from a predefined number of individual samples (e.g., 3, 5 or 10) are

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169 combined and the mixture is subsequently tested. If the mixture, or pool, tests 'Negative' then all of the
170 individual samples making up the pool are considered 'Negative.' However, if the pool is 'Positive' then
171 each of the samples making up that particular mixture must be tested individually. The concept of
172 specimen pooling has been applied in the past for large-scale screening of other infectious diseases (e.g.,
173 HIV, Hepatitis B virus) (15, 16) and has been shown to increase testing capacity and reduce reagent use
174 and expenses. A number of recent studies have assessed the potential of specimen pooling for SARS-
175 CoV-2 PCR testing and have demonstrated similar findings. Abdalhamid *et al* evaluated the performance
176 of PCR using experimental specimen pools consisting of aliquots from 3 to 10 individual samples (17).
177 This group demonstrated that the qualitative detection of SARS-CoV-2 RNA was not impacted in any of
178 the 25 specimen pools (i.e., all expected positive pools tested positive) with a maximum pool size of 5
179 individual samples. However, the C_t value of the pooled specimens increased by as much as 5.03
180 compared to the individual sample result. Similarly, Wacharapluesadee et al (18) showed that pooling
181 did not impact the sensitivity of detecting SARS-CoV-2 RNA when the PCR C_t value of an individual
182 sample was <35. However, 2 (13.3%) of 15 pools consisting of positive samples with a C_t >35 tested
183 falsely-negative. Both of these studies estimated significant improvements in testing efficiency and
184 reductions in cost when pooling is applied in a low prevalence setting (e.g., <10%) and when pools
185 consist of less than 10 samples.

186 Despite these potential advantages, a number of important factors should be considered prior
187 to implementing a specimen pooling strategy for SARS-CoV-2 testing. First, as published studies have
188 confirmed, specimen pooling increases the likelihood of low-level positive samples (i.e., those with high
189 C_t values) going undetected. As underscored above, it may be premature to conclude that a positive
190 sample with a C_t >35 is insignificant. Second, many respiratory samples testing positive for SARS-CoV-2
191 contain high amounts of viral RNA (e.g., as evidenced by associated C_t values <20), and therefore, pose a
192 significant risk to the laboratory for specimen and/or amplicon contamination. Although pooling can

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193 increase testing capacity, it requires samples to be manipulated (e.g., uncapped, pipetted) on a number
194 of occasions, thereby increasing the potential of a contamination event. Third, any manipulation of
195 clinical samples may increase the incidence of sample labeling and/or reporting errors. For example, if a
196 laboratory performing 2,500 individual tests/day implements a protocol allowing for pooling of 4
197 specimens, that laboratory could theoretically increase testing capacity to nearly 10,000 per day. Even at
198 low disease prevalence, 'de-coupling' of tens (or hundreds) of positive pools may result in reporting
199 errors. Finally, it remains unclear how billing should be handled in situations where specimen pooling is
200 applied. It is unlikely that laboratories will be reimbursed for multiple tests on a single sample (i.e., a
201 positive pool AND a subsequent test on the individual samples), and therefore, this will need to be
202 carefully considered.
203
204 ***Author's opinion***: Specimen pooling represents an option to improve efficiency and reduce costs;
205 however, it should only be considered when the testing demand for an individual laboratory far
206 outstrips the resources available to perform testing on individual samples. If specimen pooling is
207 pursued, pool sizes should be kept as small as possible (e.g., ≤5) and automated solutions for sample
208 pipetting and specimen identification used, whenever possible, to reduce the risk of contamination and
209 reporting errors.
210
211 ***Research staff performing COVID-19 clinical testing***
212 An additional strategy that has been discussed to address the increasing demand for testing has been to
213 redeploy personnel in research laboratories to COVID-19 testing. Obviously, there are thousands of
214 highly trained research scientists worldwide who have extensive expertise in performing and
215 troubleshooting molecular and serologic tests. Therefore, given the shortage of certified medical
216 laboratory scientists, it is plausible to propose that those working in research laboratories could be

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217 utilized for clinically-related COVID-19 testing. However, there are a number of important factors to
218 consider prior to pursuing this option. First, there are a number of regulations requiring that a
219 laboratory, and the staff working within it, be certified to perform testing on human specimens when
220 results are used for clinical diagnosis and management (19, 20). Specifically, the Clinical Laboratory
221 Improvement Amendments of 1988 (CLIA) state that laboratories performing non-waived testing are
222 "subject to inspection, and must meet the CLIA quality system standards, such as those for proficiency
223 testing, quality control and assessment, and personnel requirements" (19). In addition, the College of
224 American Pathologists General Checklist specifies that all personnel performing moderate- or high-
225 complexity clinical testing must meet minimum requirements, including completion of a certified clinical
226 laboratory sciences training program or at least 3 months of documented laboratory training in the
227 specialty where the individual will be working. Furthermore, the individual must demonstrate and
228 maintain competency for all testing they will perform within the clinical laboratory (20).

229 These regulations are in place to ensure that a clinical laboratory is performing testing at a
230 required standard, and most importantly, reporting accurate results for patient diagnosis and
231 management. This is an essential component of the clinical laboratory profession, and ensuring high
232 quality test results is as important – if not more important – during a pandemic as it is during normal
233 times. That being said, the COVID-19 pandemic has highlighted a key vulnerability within the diagnostic
234 community, that being a shortage in the number of trained, certified personnel to perform clinical
235 testing. We must rapidly respond to address this gap, potentially by establishing a clinical laboratory
236 'National Guard' as proposed by Bertuzzi and Patel
237 (https://www.nytimes.com/2020/04/27/opinion/biomedical-national-guard-covid.html). Doing so
238 would help ensure there is an infrastructure in place to provide clinical laboratories with the necessary
239 staff that are trained and deemed competent to provide essential testing services during a public health
240 emergency such as COVID-19. Until this is available, there are opportunities to utilize the assistance of

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241 research staff and other volunteers aside from clinical testing, including the preparation of sterile
242 aliquots of transport media, routine decontamination of the clinical laboratory and providing technical
243 guidance and troubleshooting recommendations when testing issues arise
244 (https://www.pnas.org/content/pnas/117/18/9656.full.pdf).
245
246 ***Author's opinion***: Personnel from research laboratories should not be utilized for clinical testing unless
247 they have met the required criteria to perform moderate- to high-complexity testing as outlined by CLIA
248 and other regulatory agencies. Although research scientists are highly trained with extraordinary
249 expertise, enlisting their service in a clinical laboratory prior to meeting these requirements sets a
250 dangerous precedent for the clinical laboratory profession.
251
252 ***Should everyone who wants a test get a test?***
253 In recent years, Clinical Microbiologists have made a concerted effort to promote the judicious use of
254 laboratory tests, emphasizing the importance of stewardship and data-driven decision-making. Many
255 diagnostics experts have been actively involved in the creation of testing algorithms and clinical decision
256 support tools, which guide providers to order the most appropriate tests, assist with result
257 interpretation and inform follow-up testing recommendations (21, 22). These efforts have been
258 necessary in order to quell the rising costs of healthcare and ensure that limited resources are used
259 wisely. Despite these efforts, testing for SARS-CoV-2 has been applied in the asymptomatic population
260 and unnecessary repeat molecular testing is common. This has led to a significant detrimental impact on
261 the global supply chain, delays in result turnaround time, and most importantly, a shortage of tests for
262 those who need testing most.
263 Recently, there has been discussion of the potential merits of testing a large percentage of the
264 population with rapid and inexpensive at-home assays (23). The concept proposes testing asymptomatic

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265 individuals with high frequency (e.g., every few days), which may counter-balance lower sensitivity.
266 While interesting in theory, this approach is unlikely to be feasible and may be problematic. Consider,
267 for example, the strategy of testing students in the United States in order for them to attend school.
268 According to the National Center for Education Statistics
269 (https://nces.ed.gov/fastfacts/display.asp?id=372), there are ~56 million students attending elementary,
270 middle and high schools in the U.S. Even if 25% of those students were tested regularly (e.g., three times
271 each week), that would equate to 42 million tests per week (i.e., 756 million tests between September 1
272 and December 31, 2020). Assuming an overall disease prevalence of 5% and a screening assay with 98%
273 specificity, nearly 800,000 false-positive results would occur weekly (i.e., >14 million false-positive
274 results by year's end). False-positive results for SARS-CoV-2 are not inconsequential, as they may lead to
275 loss of work, separation from family members and unnecessary psychological distress. Furthermore,
276 there is no reason to assume that rapid, at-home tests will be immune to the same supply chain
277 challenges that have plagued clinical laboratories during the COVID-19 pandemic.
278
279 ***Author's opinion***: At this stage of the pandemic, a "test everyone" strategy is unlikely to be feasible and
280 will prevent access of limited resources to those who need testing most. Testing should be prioritized for
281 those situations where the result will inform patient management, personal protective equipment use
282 and isolation decisions.
283
284 **Summary**
285 The COVID-19 pandemic has presented a significant challenge to the diagnostic community; however,
286 the valiant efforts of clinical laboratory professionals, public health and industry partners have made a
287 tremendous impact on improving the diagnosis and management of infected individuals and reducing
288 the spread of disease. Given that the virus is now well-entrenched in the United States, a successful end

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289 to the outbreak will require the thoughtful application of testing, consistent/universal masking policies
290 and continued physical distancing measures. As a profession, clinical microbiologists should continue to
291 promote diagnostic stewardship and its importance during a pandemic. In addition, we should highlight
292 the COVID-19 pandemic as an example of why the rapid development and implementation of diagnostic
293 assays is necessary to prevent the spread of future novel infectious diseases.
294

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