AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

COMMENTARY

Challenges and Controversies to Testing for COVID-19

Matthew J. Binnicker[a,b]

[a]Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA
[b]Director of Clinical Virology, Mayo Clinic, Rochester, Minnesota, USA

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

The coronavirus disease (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus (SARS-CoV-2), has been confirmed in nearly 20 million cases and caused over 730,000 deaths worldwide (https://coronavirus.jhu.edu). As the virus has spread across the globe, laboratory testing has played an important role in diagnosing those with disease, as well as identifying individuals who are asymptomatic yet have the potential to serve as a source of viral transmission. Molecular tests, such as real-time PCR, have been the most common laboratory tool used to detect cases of COVID-19. In fact, SARS-CoV-2 molecular assays have become an integral component in a multipronged strategy aimed at reducing transmission of the virus. This strategy has consisted of (i) case identification (i.e., through testing), (ii) quarantine or isolation of exposed/infected individuals, and (iii) contact tracing. Despite the broad application of this strategy, cases of COVID-19 have continued to surge, especially in the United States, where the number of confirmed infections has surpassed 5 million (~25% of the global case count).

The COVID-19 pandemic is an unprecedented health care crisis that has required clinical and laboratory professionals to rapidly adapt to new information, innovate, and, in some situations, implement practices that would not be considered under normal circumstances. The emergence of SARS-CoV-2 has forced clinical laboratories and test manufacturers to develop novel diagnostic assays in a time frame that previously would not have been considered feasible. As testing options have become available, health care professionals and diagnostics experts have had to learn how to best apply these tools to diagnose and manage patients with COVID-19 and slow the spread of disease. Some of these lessons have shown routine testing approaches to be effective, while

Citation Binnicker MJ. 2020. Challenges and controversies to testing for COVID-19. J Clin Microbiol 58:e01695-20. https://doi.org/10.1128/JCM.01695-20.

Editor Colleen Suzanne Kraft, Emory University

Address correspondence to binnicker.matthew@mayo.edu.

Accepted manuscript posted online 12 August 2020
Published 21 October 2020

others have taught us that a new pathogen, such as SARS-CoV-2, may require a new diagnostic playbook.

As SARS-CoV-2 has spread globally, it has required laboratory professionals to venture into uncharted territory. Not only is testing being used to diagnose patients with symptomatic disease, it is being incorporated into local, state, and federal strategies to reopen the economy. Businesses, schools, and even athletic teams are considering how laboratory testing can be used to demonstrate that asymptomatic employees, customers, students, and athletes are safe to return to normal activities. Some hospitals and clinics are utilizing SARS-CoV-2 testing prior to certain procedures and surgeries to reduce nosocomial transmission and prevent poor outcomes in high-risk patients. This trend toward large-scale screening of asymptomatic individuals has placed an incredible burden on the global testing infrastructure and has created challenges with regard to how testing should be utilized and results interpreted. Therefore, it is not surprising that a number of diagnostic uncertainties and controversies have arisen. In this commentary, I will address several topics that have been the focus of continued discussion and debate, including (i) the clinical sensitivity of molecular assays and their role in defining the duration of quarantine/isolation, (ii) whether the PCR cycle threshold value should be included on patient reports, (iii) the potential for specimen pooling and testing by research staff to expand testing capacity, and (iv) whether testing a large percentage of the population represents a viable strategy to end the pandemic.

CLINICAL SENSITIVITY OF PCR AND ITS USE AS A TEST OF CURE

Molecular tests, such as real-time PCR, have become a cornerstone in the diagnosis of infectious diseases and have been the most common laboratory method utilized during the COVID-19 pandemic. The inherent sensitivity of real-time PCR allows for detection of minute amounts (e.g., $<$100 copies/ml) of target nucleic acid in clinical samples. Despite this, one of the earliest challenges arising during the COVID-19 pandemic was the concern that cases were going undetected by real-time PCR (1–3). Several studies reported the clinical sensitivity of SARS-CoV-2 real-time PCR assays performed on upper respiratory swab samples to be in the range of 60 to 70% (4, 5). However, as experience was gained with testing and additional data published, it was recognized that the likelihood of detecting SARS-CoV-2 RNA is highly dependent on the timing of sample collection, the type of specimen that is obtained, and the quality of the sample. SARS-CoV-2 is present at the largest amounts in the upper respiratory tract (URT) (i.e., the nasopharynx) during the first several days following symptom onset (typically 5 to 7 days following exposure) and subsequently declines in the URT over the course of the following week (6). During the later stages of disease (e.g., $>$7 days postonset of symptoms), lower respiratory tract (LRT) samples, such as sputum, bronchoalveolar lavage (BAL) fluid, or tracheal secretions, may yield higher rates of detection (4). Due to these nuances, it has been challenging for laboratory professionals to truly define the clinical sensitivity of SARS-CoV-2 real-time PCR and has required that negative results be interpreted in the context of the timing of sample collection (early postonset versus late postonset), the type of specimen tested (e.g., nasopharyngeal [NP] swab versus throat swab), and the performance characteristics of the assay.

Despite the observation that some COVID-19 patients initially test negative by real-time PCR (1, 2), a dichotomous observation has been the persistent detection of SARS-CoV-2 RNA in other patients (7). A number of published reports have demonstrated that SARS-CoV-2 real-time PCR assays may be positive for weeks, even following the resolution of clinical symptoms (7, 8). The author is aware of a case where a patient with an underlying health condition tested positive by PCR for over 100 days (unpublished data). Many health care institutions have followed a test-based strategy—initially recommended by the U.S. Centers for Disease Control and Prevention (CDC)—in which two negative PCR results (obtained on serial samples collected at least 24 h apart) were required prior to releasing a patient from isolation. This approach, whereby qualitative molecular assays have been used to assess whether an individual is infectious, has led

to prolonged isolation, loss of work, extended use of personal protective equipment for hospitalized patients, and psychological distress for patients and their family members.

Laboratory professionals have recognized for years that qualitative molecular assays for infectious diseases can remain positive following the resolution of disease, and therefore, these methods are not typically recommended as "tests of cure" (9, 10). Due to this limitation, the U.S. CDC no longer recommends the use of a SARS-CoV-2 test-based strategy to determine when to discontinue transmission-based precautions, instead relying on a symptom-based strategy in the majority of situations (https://www.cdc.gov/coronavirus/2019-ncov/hcp/duration-isolation.html).

Author's opinion. Molecular methods, including real-time PCR, should not be used following an initial diagnosis of COVID-19 to determine whether an individual continues to shed infectious SARS-CoV-2. Repeat molecular testing may be indicated in patients who recover and subsequently develop new COVID-19-related symptoms.

PROVIDING THE PCR CYCLE THRESHOLD VALUE IN THE PATIENT REPORT

Due to the possibility that SARS-CoV-2 molecular assays can remain persistently reactive in patients who have recovered from COVID-19 or remain asymptomatic, there has been interest in whether semiquantitative or quantitative data can assist in result interpretation. For many commercial and laboratory-developed real-time PCR assays, the cycle threshold (C_T) value associated with a PCR result is available to the laboratory staff. The C_T value is inversely proportional to the amount of target nucleic acid and can be used as a relative indicator of the concentration of a pathogen in a clinical specimen. For example, a positive PCR result with an associated C_T value of 15 would indicate a very high concentration of the target nucleic acid in a sample, whereas a C_T of 35 may suggest that the target is present but near the assay's limit of detection. Importantly, the C_T value is nonnormalized and, therefore, cannot be considered a quantitative result. This is because the C_T value is dependent on a number of variables, including the assay's gene target, the extraction platform, PCR amplification chemistry, and even the quality of specimen collection.

Despite these limitations, several studies have assessed whether a correlation can be made between the PCR C_T value and the presence of replication-competent virus by using viral culture as a surrogate for a patient's infectious status. Bullard et al. (11) compared viral culture and C_T values for 90 respiratory samples that were positive by PCR and demonstrated that a $C_T > 24$ showed a strong correlation with reduced recovery of SARS-CoV-2 in cell culture. Similarly, La Scola et al. inoculated 183 PCR positive respiratory samples in a Vero E6 cell line (12). This group demonstrated a similar reduction in culture positivity as the C_T value increased; however, in this study, a $C_T > 34$ was proposed as a threshold to estimate that an individual is no longer shedding infectious virus. Unfortunately, PCR C_T values may vary significantly between assays, even those using the same gene target (13, 14). During the COVID-19 pandemic, it has become common for clinical laboratories to perform multiple real-time PCR assays to detect SARS-CoV-2. Therefore, including the C_T value on all positive results may be confusing and misleading to ordering providers. Since the C_T value is not normalized against a human gene internal control, it is possible that a high C_T value (e.g., >30) could be due to an inadequate sample collection rather than a low level of target nucleic acid or "noninfectious" virus. Additional data are needed before a definitive viral load threshold correlating with infectivity can be established. Finally, we should be cautious to equate viral culture negativity with an individual's inability to serve as a source of viral transmission. Due to inferior sensitivity, viral culture has been replaced by molecular assays for the diagnosis of a number of respiratory viral infections.

Author's opinion. The PCR C_T value for qualitative SARS-CoV-2 real-time PCR assays should not be routinely reported. On a case-by-case basis, the C_T value may be provided (i.e., verbally) to the ordering physician upon request. This approach allows for clarification of the assay used for testing and a discussion of the limitations associated with using the C_T value while interpreting the result.

SPECIMEN POOLING AS AN APPROACH TO INCREASE TESTING CAPACITY

One of the most challenging aspects of the COVID-19 pandemic for clinical laboratories has been ongoing supply chain shortages and disruptions. Laboratories have not only struggled with maintaining an adequate supply of test reagents, but also other essential supplies, including consumables (e.g., pipette tips, 96-well plates), swabs, and viral transport media. As the demand for testing has increased alongside a global shortage of necessary supplies, laboratory professionals have been forced to identify ways to "do more with less." One potential solution receiving a significant amount of attention is specimen pooling, whereby aliquots from a predefined number of individual samples (e.g., 3, 5, or 10) are combined and the mixture is subsequently tested. If the mixture, or pool, tests negative, then all of the individual samples making up the pool are considered negative. However, if the pool is positive, then each of the samples making up that particular mixture must be tested individually. The concept of specimen pooling has been applied in the past for large-scale screening of other infectious diseases (e.g., HIV, hepatitis B virus) (15, 16) and has been shown to increase testing capacity and reduce reagent use and expenses. A number of recent studies have assessed the potential of specimen pooling for SARS-CoV-2 PCR testing and have demonstrated similar findings. Abdalhamid et al. evaluated the performance of PCR using experimental specimen pools consisting of aliquots from 3 to 10 individual samples (17). This group demonstrated that the qualitative detection of SARS-CoV-2 RNA was not impacted in any of the 25 specimen pools (i.e., all expected positive pools tested positive) with a maximum pool size of 5 individual samples. However, the C_T value of the pooled specimens increased by as much as 5.03 compared to the individual sample result. Similarly, Wacharapluesadee et al. (18) showed that pooling did not impact the sensitivity of detecting SARS-CoV-2 RNA when the PCR C_T value of an individual sample was <35. However, 2 (13.3%) of 15 pools consisting of positive samples with a C_T >35 tested falsely negative. Both of these studies estimated significant improvements in testing efficiency and reductions in cost when pooling is applied in a low-prevalence setting (e.g., $<10\%$) and when pools consist of less than 10 samples.

Despite these potential advantages, a number of important factors should be considered prior to implementing a specimen pooling strategy for SARS-CoV-2 testing. First, as published studies have confirmed, specimen pooling increases the likelihood of low-level positive samples (i.e., those with high C_T values) going undetected. As underscored above, it may be premature to conclude that a positive sample with a C_T >35 is insignificant. Second, many respiratory samples testing positive for SARS-CoV-2 contain large amounts of viral RNA (e.g., as evidenced by associated C_T values <20) and, therefore, pose a significant risk to the laboratory for specimen and/or amplicon contamination. Although pooling can increase testing capacity, it requires samples to be manipulated (e.g., uncapped or pipetted) on a number of occasions, thereby increasing the potential of a contamination event. Third, any manipulation of clinical samples may increase the incidence of sample labeling and/or reporting errors. For example, if a laboratory performing 2,500 individual tests/day implements a protocol allowing for pooling of 4 specimens, that laboratory could theoretically increase testing capacity to nearly 10,000 per day. Even at low disease prevalence, "decoupling" of tens (or hundreds) of positive pools may result in reporting errors. Finally, it remains unclear how billing should be handled in situations where specimen pooling is applied. It is unlikely that laboratories will be reimbursed for multiple tests on a single sample (i.e., a positive pool and a subsequent test on the individual samples), and therefore, this will need to be carefully considered.

Author's opinion. Specimen pooling represents an option to improve efficiency and reduce costs; however, it should only be considered when the testing demand for an individual laboratory far outstrips the resources available to perform testing on individual samples. If specimen pooling is pursued, pool sizes should be kept as small as possible (e.g., ≤ 5) and automated solutions for sample pipetting and specimen

identification used, whenever possible, to reduce the risk of contamination and reporting errors.

RESEARCH STAFF PERFORMING COVID-19 CLINICAL TESTING

An additional strategy that has been discussed to address the increasing demand for testing has been to redeploy personnel in research laboratories to COVID-19 testing. Obviously, there are thousands of highly trained research scientists worldwide who have extensive expertise in performing and troubleshooting molecular and serologic tests. Therefore, given the shortage of certified medical laboratory scientists, it is plausible to propose that those working in research laboratories could be utilized for clinically related COVID-19 testing. However, there are a number of important factors to consider prior to pursuing this option. First, there are a number of regulations requiring that a laboratory, and the staff working within it, be certified to perform testing on human specimens when results are used for clinical diagnosis and management (19, 20). Specifically, the Clinical Laboratory Improvement Amendments of 1988 (CLIA) state that laboratories performing nonwaived testing are "subject to inspection, and must meet the CLIA quality system standards, such as those for proficiency testing, quality control and assessment, and personnel requirements" (19). In addition, the College of American Pathologists General Checklist specifies that all personnel performing moderate- or high-complexity clinical testing must meet minimum requirements, including completion of a certified clinical laboratory sciences training program or at least 3 months of documented laboratory training in the specialty where the individual will be working. Furthermore, the individual must demonstrate and maintain competency for all testing they will perform within the clinical laboratory (20).

These regulations are in place to ensure that a clinical laboratory is performing testing at a required standard and, most importantly, reporting accurate results for patient diagnosis and management. This is an essential component of the clinical laboratory profession, and ensuring high-quality test results is as important—if not more important—during a pandemic as it is during normal times. That being said, the COVID-19 pandemic has highlighted a key vulnerability within the diagnostic community, that being a shortage in the number of trained, certified personnel to perform clinical testing. We must rapidly respond to address this gap, potentially by establishing a clinical laboratory "national guard" as proposed by Bertuzzi and Patel (https://www.nytimes.com/2020/04/27/opinion/biomedical-national-guard-covid.html). Doing so would help ensure there is an infrastructure in place to provide clinical laboratories with the necessary staff that are trained and deemed competent to provide essential testing services during a public health emergency such as COVID-19. Until this is available, there are opportunities to utilize the assistance of research staff and other volunteers aside from clinical testing, including the preparation of sterile aliquots of transport media, routine decontamination of the clinical laboratory, and providing technical guidance and troubleshooting recommendations when testing issues arise (https://www.pnas.org/content/pnas/117/18/9656.full.pdf).

Author's opinion. Personnel from research laboratories should not be utilized for clinical testing unless they have met the required criteria to perform moderate- to high-complexity testing as outlined by CLIA and other regulatory agencies. Although research scientists are highly trained with extraordinary expertise, enlisting their service in a clinical laboratory prior to meeting these requirements sets a dangerous precedent for the clinical laboratory profession.

SHOULD EVERYONE WHO WANTS A TEST GET A TEST?

In recent years, clinical microbiologists have made a concerted effort to promote the judicious use of laboratory tests, emphasizing the importance of stewardship and data-driven decision-making. Many diagnostics experts have been actively involved in the creation of testing algorithms and clinical decision support tools, which guide providers to order the most appropriate tests, assist with result interpretation, and inform follow-up testing recommendations (21, 22). These efforts have been necessary

in order to quell the rising costs of health care and ensure that limited resources are used wisely. Despite these efforts, testing for SARS-CoV-2 has been applied in the asymptomatic population, and unnecessary repeat molecular testing is common. This has led to a significant detrimental impact on the global supply chain, delays in result turnaround time, and, most importantly, a shortage of tests for those who need testing most.

Recently, there has been discussion of the potential merits of testing a large percentage of the population with rapid and inexpensive at-home assays (23). The concept proposes testing asymptomatic individuals with high frequency (e.g., every few days), which may counterbalance lower sensitivity. While interesting in theory, this approach is unlikely to be feasible and may be problematic. Consider, for example, the strategy of testing students in the United States in order for them to attend school. According to the National Center for Education Statistics (https://nces.ed.gov/fastfacts/display.asp?id=372), there are ~56 million students attending elementary, middle, and high schools in the United States. Even if 25% of those students were tested regularly (e.g., three times each week), that would equate to 42 million tests per week (i.e., 756 million tests between 1 September and 31 December 2020). Assuming an overall disease prevalence of 5% and a screening assay with 98% specificity, nearly 800,000 false-positive results would occur weekly (i.e., >14 million false-positive results by year's end). False-positive results for SARS-CoV-2 are not inconsequential, as they may lead to loss of work, separation from family members, and unnecessary psychological distress. Furthermore, there is no reason to assume that rapid at-home tests will be immune to the same supply chain challenges that have plagued clinical laboratories during the COVID-19 pandemic.

Author's opinion. At this stage of the pandemic, a "test everyone" strategy is unlikely to be feasible and will prevent access of limited resources to those who need testing most. Testing should be prioritized for those situations where the result will inform patient management, personal protective equipment use, and isolation decisions.

SUMMARY

The COVID-19 pandemic has presented a significant challenge to the diagnostic community; however, the valiant efforts of clinical laboratory professionals and public health and industry partners have made a tremendous impact on improving the diagnosis and management of infected individuals and reducing the spread of disease. Given that the virus is now well entrenched in the United States, a successful end to the outbreak will require the thoughtful application of testing, consistent/universal masking policies, and continued physical distancing measures. As a profession, clinical microbiologists should continue to promote diagnostic stewardship and its importance during a pandemic. In addition, we should highlight the COVID-19 pandemic as an example of why the rapid development and implementation of diagnostic assays are necessary to prevent the spread of future novel infectious diseases.

REFERENCES

1. Cao G, Tang S, Yang D, Shi W, Wang X, Wang H, Li C, Wei J, Ma L. 2020. The potential transmission of SARS-CoV-2 from patients with negative RT-PCR swab tests to others: two related clusters of COVID-19 outbreak. Jpn J Infect Dis https://doi.org/10.7883/yoken.JJID.2020.165.
2. Chen L, Li H, Ye Y, Wu Z, Huang Y, Zhang W, Lin L. 2020. A COVID-19 patient with multiple negative results for PCR assays outside Wuhan, China: a case report. BMC Infect Dis 20:517. https://doi.org/10.1186/s12879-020-05245-7.
3. Valent F, Doimo A, Mazzini G, Pipan C. 2020. RT-PCR tests for SARS-CoV-2 processed at a large Italian hospital and false-negative results among confirmed COVID-19 cases. Infect Control Hosp Epidemiol 11:1–2. https://doi.org/10.1017/ice.2020.290.
4. Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, Tan W. 2020. Detection of SARS-CoV-2 in different types of clinical specimens. JAMA 323:1843–1844. https://doi.org/10.1001/jama.2020.3786.
5. Ai T, Yang Z, Hou H, Zhan C, Chen C, Li W, Tao Q, Sun Z, Xia L. 2020. Correlation of chest CT and RT-PCR testing for coronavirus disease 2019 (COVID-19) in China: a report of 1014 cases. Radiology 296:E32–E40. https://doi.org/10.1148/radiol.2020200642.
6. He X, Lau EHY, Wu P, Deng X, Wang J, Hao X, Lau YC, Wong JY, Guan Y, Tan X, Mo X, Chen Y, Liao B, Chen W, Hu F, Zhang Q, Zhong M, Wu Y, Zhao L, Zhang F, Cowling BJ, Li F, Leung GM. 2020. Temporal dynamics in viral shedding and transmissibility of COVID-19. Nat Med 26:672–675. https://doi.org/10.1038/s41591-020-0869-5.
7. Cargo A, Pereira-Vaz J, Mota V, Mendez A, Morais C, Coelho da Silva A, Camilo E, Pinto CS, Cunha E, Pereira J, Coucelo M, Martinho P, Correa L, Marques G, Araujo L, Rodrigues F. 2020. Clearance and persistence of SARS-CoV-2 RNA in patients with COVID-19. J Med Virol https://doi.org/10.1002/jmv.26103.
8. Cevik M, Tate M, Lloyd O, Maraolo AE, Schafers J, Ho A. 2020. SARS-CoV-2 viral load dynamics, duration of viral shedding and infectiousness:

a living systematic review and meta-analysis. medRxiv https://www.medrxiv.org/content/10.1101/2020.07.25.20162107v2.
9. Bagdasarian N, Rao K, Malani PN. 2015. Diagnosis and treatment of *Clostridium difficile* in adults: a systematic review. JAMA 313:398–408. https://doi.org/10.1001/jama.2014.17103.
10. Jartti T, Lehtinen P, Vuorinen T, Koskenvuo M, Ruuskanen O. 2004. Persistence of rhinovirus and enterovirus RNA after acute respiratory illness in children. J Med Virol 72:695–699. https://doi.org/10.1002/jmv.20027.
11. Bullard J, Dust K, Funk D, Strong JE, Alexander D, Garnett L, Boodman C, Bello A, Hedley A, Schiffman Z, Dona K, Bastien N, Li Y, Van Caeseele PG, Poliquin G. 2020. Predicting infectious SARS-CoV-2 from diagnostic samples. Clin Infect Dis https://doi.org/10.1093/cid/ciaa638.
12. La Scola B, Le Bideau M, Andreani J, Hoang VT, Grimaldier C, Colson P, Gautret P, Raoult D. 2020. Viral RNA load as determined by cell culture as a management tool for discharge of SARS-CoV-2 patients from infectious disease wards. Eur J Clin Microbiol Infect Dis 39:1059–1061. https://doi.org/10.1007/s10096-020-03913-9.
13. Nalla AK, Casto AM, Huang M-LW, Perchetti GA, Sampoleo R, Shrestha L, Wei Y, Zhu H, Jerome KR, Greninger AL. 2020. Comparative performance of SARS-CoV-2 detection assays using seven different primer-probe sets and one assay kit. J Clin Microbiol 58:e00557-20. https://doi.org/10.1128/JCM.00557-20.
14. Rodino KG, Espy MJ, Buckwalter SP, Walchak RC, Germer JJ, Fernholz E, Boerger A, Schuetz AN, Yao JD, Binnicker MJ. 2020. Evaluation of saline, phosphate-buffered saline, and minimum essential medium as potential alternatives to viral transport media for SARS-CoV-2 testing. J Clin Microbiol 58:e00590-20. https://doi.org/10.1128/JCM.00590-20.
15. Dinesha TR, Boobalan J, Sivamalar S, Subashini D, Solomon SS, Murugavel KG, Balakrishnan P, Smith DM, Saravanan S. 2018. Occult HBV infection in HIV-infected adults and evaluation of pooled NAT for HBV. J Viral Hepat 25:718–723. https://doi.org/10.1111/jvh.12858.
16. Boobalan J, Dinesha TR, Gomathi S, Elakkiya E, Pradeep A, Chitra D, Murugavel KG, Balakrishnan P, Shantha S, Solomon SS, Kumarasamy N, Smith DM, Saravanan S. 2019. Pooled nucleic acid testing strategy for monitoring HIV-1 treatment in resource limited settings. J Clin Virol 117:56–60. https://doi.org/10.1016/j.jcv.2019.05.012.
17. Abdalhamid B, Bilder CR, McCutchen EL, Hinrichs S, Koepsell SA, Iwen PC. 2020. Assessment of specimen pooling to conserve SARS-CoV-2 testing resources. Am J Clin Pathol 153:715–718. https://doi.org/10.1093/ajcp/aqaa064.
18. Wacharapluesadee S, Kaewpom T, Ampoot W, Ghai S, Khamhang W, Worachotsueptrakun K, Wanthong P, Nopvichai C, Supharatpariyakorn T, Putcharoen O, Paitoonpong L, Suwanpimolkul G, Jantarabenjakul W, Hemachudha P, Krichphiphat A, Buathong R, Plipat T, Hemachudha T. 2020. Evaluating the efficiency of specimen pooling for PCR-based detection of COVID-19. J Med Virol https://doi.org/10.1002/jmv.26005.
19. Code of Federal Regulations. 2020. Title 42. Public Health. Chapter IV. Centers for Medicare & Medicaid Services, Department Of Health And Human Services. Subchapter G. Standards and certification. Part 493. Laboratory requirements. Subpart A. General provisions. https://www.ecfr.gov/cgi-bin/text-idx?SID=1248e3189da5e5f936e55315402bc38b&node=pt42.5.493&rgn=div5. Accessed 17 August 2020.
20. College of American Pathologists. 2020. Laboratory general checklist. GEN.54750, nonwaived testing personnel qualifications, p 79. College of American Pathologists, Northfield, IL.
21. Miller JM, Binnicker MJ, Campbell S, Carroll KC, Chapin KC, Gilligan PH, Gonzalez MD, Jerris RC, Kehl SC, Patel R, Pritt BS, Richter SS, Robinson-Dunn B, Schwartzman JD, Snyder JW, Telford S, Theel ES, Thomson RB, Weinstein MP, Yao JD. 2018. A guide to utilization of the microbiology laboratory for diagnosis of infectious diseases: 2018 update by the Infectious Diseases Society of America and the American Society for Microbiology. Clin Infect Dis 67:e1–e94. https://doi.org/10.1093/cid/ciy381.
22. Patel R, Babady E, Theel ES, Storch GA, Pinsky BA, St George K, Smith TC, Bertuzzi S. 2020. Report from the American Society for Microbiology COVID-19 International Summit, 23 March 2020: value of diagnostic testing for SARS-CoV-2/COVID-19. mBio 11:e00722-20. https://doi.org/10.1128/mBio.00722-20.
23. Larremore DB, Wilder B, Lester E, Shehata S, Burke JM, Hay JA, Tambe M, Mina MJ, Parker R. 2020. Test sensitivity is secondary to frequency and turnaround time for COVID-19 surveillance. medRxiv https://www.medrxiv.org/content/10.1101/2020.06.22.20136309v2.