

REVIEW



Quantifying SARS-CoV-2 viral load: current status and future prospects

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ABSTRACT

Introduction: The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) emerged as a novel viral agent that quickly spread worldwide. SARS-CoV-2 is responsible for the human coronavirus disease 2019 (COVID-19) which has claimed hundreds of thousands of lives and had an immeasurable toll on the economy. Currently, most clinical cases are identified by qualitative molecular testing. However, the need for quantitative assessment is gaining traction.

Areas covered: In this review, the current state and future perspective of SARS-CoV-2 viral load quantification is presented.

Expert opinion: Viral load quantification is a critical measure that informs clinicians of treatment response, actionable viral load levels, and guidance on patient management. Additionally, for pathogens with epidemiological consequences, viral load can provide information to guide infection control measures and policies. While qualitative detection is sufficient to identify cases and initiate containment and mitigation measures in the vast majority of COVID-19 cases, in certain situations, SARS-CoV-2 quantification is needed to assess viral load trending. However, there are obstacles to quantification, including variability in respiratory specimen collection and the lack of commutable reference material. At the same time, the need for quantification for clinical and epidemiological management is growing, especially concerning individuals with prolonged RNA shedding.

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1. Introduction

The respiratory viral infection human coronavirus disease 2019 (COVID-19), caused by a novel betacoronavirus named severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), initially reported in December 2019 in Hubei province, China, spread across the globe within a few weeks, paralyzing the world in an unprecedented way [1–6]. Like pandemics in the past, the exact origins and first human transmission events of SARS-CoV-2 remain a mystery [7]. As of this writing, COVID-19 pandemic has claimed the lives of close to three million people worldwide [8]. Also devastating are the tens of millions of patients admitted to intensive care units in isolation commonly referred to as COVID wards, where a vast number of them would be put on ventilators and rigorously managed. For survivors of COVID-19, the sequelae of the disease is not fully known and still evolving, but it is clear that many who have recovered still need long-term management from complications of COVID-19 [9]. This includes cardiovascular and neurological manifestations, as well as continuous pulmonary function abnormalities [9]. In fact, academic medical institutions across the United States are establishing post-COVID-19 care centers to manage patients with late sequelae [9]. As with any highly transmissible infection, rapid identification of infected individuals with effective isolation or quarantine measures are paramount to controlling the spread. In order to provide rapid diagnosis, governments and test manufacturers around the world were under high pressure in the early days of the pandemic to produce fast and accurate diagnostic tests.

Due to their efforts, the field saw an explosion of SARS-CoV-2 tests, although test reagents and consumables needed to perform the tests were not always in adequate supply [10]. As our infrastructure continue to adapt to the pandemic, as variants emerge, and vaccines are more widely rolled out, COVID-19 testing strategies also need to evolve, including the need for reliable quantitative tests to assess viral load. In this review, the current state and future perspective of SARS-CoV-2 viral load quantification is presented. Due to the complexities around regulatory requirements across jurisdictions, the focus of this review centers around the United States. However, approaches to testing and technological advancements or limitations are applicable to any jurisdiction.

2. Clinical tests to diagnose COVID-19

In the United States, the Food and Drug Administration (FDA) regulates diagnostic test manufacturing. Other jurisdictions have similar regulatory bodies that oversee test manufacturing like the European Medicine Agency (EMA) in the European Union or National Medical Products Administration (NMPA) in China. Given the need for rapid deployment of diagnostic tests to combat the pandemic, on 4 February 2020, the US Health and Human Services declared a public health emergency concerning COVID-19 [11]. The declaration allows the FDA to authorize unapproved medical products to be used during a public health emergency, including diagnostic tests. Thus, test manufacturers can apply for an FDA Emergency Use

Table 1. Select U.S. FDA Emergency Use Authorized Tests

Assay	Method +	Targets	Specimen Type±	Collection/ Transport	Percent Positive Agreement**	Percent Negative Agreement**	Limit of Detection***	FDA Reference Panel****
BioFire COVID-19*	PCR	ORF 1ab, ORF 1ab, ORF8	Upper Respiratory	1–3 mL transport medium, saline, phosphate buffered saline	97.7% (43/44)	100% (87/87)	3.3E+02 GE/mL	5.4E+03 NDU/mL
Xpert Xpress SARS-CoV-2*	PCR	N2, E	Lower Respiratory	Sterile container	100% (15/15)	93.1% (27/29)	1.0E+04 GE/mL	N.A.
			Pooled Nasopharyngeal swab, Eight Samples	1–3 mL transport medium, saline, phosphate buffered saline	76.4–99.1% (95% C.I.)	N.A.	N.A.	N.A.
cobas SARS-CoV-2	PCR	ORF 1ab, E	Upper Respiratory	3 mL viral transport medium, saline	97.8% (44/45)	95.6% (43/45)	0.0050 PFU/mL	5.4E+03 NDU/mL
			Upper Respiratory	Universal or viral transport medium, saline	100% (30/30)	95.5% (126/132)	3.2E+01 copies/mL	1.8E+03 NDU/mL
Alinity m SARS-CoV-2	PCR	N2, RdRp	Pooled Upper Respiratory Swab, Six Samples	Universal or viral transport medium, saline	83.9–100% (95% C.I.)	N.A.	N.A.	N.A.
			Upper Respiratory	Viral transport medium	100% (47/47)	96.5% (55/57)	6.0E+02 copies/mL	6.0E+02 NDU/mL
Aptima SARS-CoV-2	TMA	ORF 1ab, ORF 1ab	Lower Respiratory	Sterile container	N.A.	N.A.	N.A.	N.A.
			Pooled Upper Respiratory Swab, Five Samples	Viral transport medium	92.3–100% (95% C.I.)	93.5–100% (95% C.I.)	N.A.	N.A.
TaqPath COVID-19 Combo Kit	PCR	ORF 1ab, S, N	Upper Respiratory	Transport medium, saline	100% (50/50)	98.2% (54/55)	8.3E+01 copies/mL	6.0E+02 NDU/mL
			Pooled Upper Respiratory Swab, Five Samples	Transport medium, saline	92.9–100% (95% C.I.)	83.9–100% (95% C.I.)	N.A.	N.A.
Simplexa COVID-19 Direct	PCR	S, ORF 1ab	Upper Respiratory	Transport medium, saline	N.A.	N.A.	1.0E+01 GE/mL	1.8E+05 NDU/mL
			Lower Respiratory	Sterile container	99.1% (111/112)	100% (116/116)	5.0E+02 GE/mL	6.0E+03 NDU/mL

*Multiplex assays combined with influenza and RSV targets are available and commonly in used

**Clinical performance, symptomatic patient samples or symptomatology unknown

***GE (genomic equivalent), PFU (plaque forming unit), NDU (NAAT Detectable Unit)

±Upper Respiratory includes nasopharyngeal, oropharyngeal, mid-turbinate, or anterior nasal swab; some assays include nasal wash/aspirate

±Lower Respiratory includes sputum (induced or expectorated), bronchoalveolar lavage fluid

± PCR (Polymerase Chain Reaction), TMA (Transcript Mediated Amplification)

Authorization (EUA) without submitting extensive data and obtaining an FDA in vitro diagnostic (IVD) approval that can take years.

As of this writing, there are over 220 EUA assays for the detection of SARS-CoV-2 RNA or antigen and over 70 EUA assays for the detection of antibody against SARS-CoV-2 [12]. These assays have various specimen requirements, as well as collection options. These figures don't include the hundreds of laboratory-developed tests (LDT) and those that are investigational or research use only that may be in use in various research and clinical settings. Table 1 lists several commonly used EUA qualitative assays and their respective test characteristics and analytical performance (Table 1). It is important to note that qualitative SARS-CoV-2 assays are slowly getting approved by the FDA. Most recently, BioFire Respiratory Viral Panel 2.1, a qualitative multiplex assay that includes SARS-CoV-2 as a target, received FDA IVD approval [13]. Once the public health emergency declaration ends, EUA assays will no longer be allowed. Therefore, it is important for manufacturers to obtain FDA IVD approval of their EUA assays prior to the end of the declaration.

The clinical use of any of these qualitative SARS-CoV-2 assays depends on the indication for testing, including onset of symptoms consistent with COVID-19, close contact exposure, or asymptomatic screening or mass surveillance. For diagnostic purposes to assess whether a person is currently infected, detection of SARS-CoV-2 RNA by a molecular method like nucleic acid amplification test (NAAT) or detection of SARS-CoV-2 antigen by an immunoassay would be most appropriate. To evaluate past-infection and understand the epidemiology of COVID-19 and assess seroprevalence, including immunization, detection of antibody against SARS-CoV-2 would be most appropriate. The pretest probabilities of these methodologies and window of detection are largely dependent upon the timing of the infection, anatomic site of sample collection, prevalence in the community or specific population, and good collection techniques [14]. A number of studies show that viral antigen is detectable by current methods within the first 5 to 7 day post-onset of symptoms [15,16]. This is a much shorter detection window compared to viral RNA detected by NAAT, which can be detectable weeks into the infection [15]. Antibody detection occurs after the second week post-infection, and optimally detectable at 3–4 weeks [15,17]. Unconventionally, both IgM and IgG seems to rise at the same time, making IgM detection not as helpful in diagnosing acute infection in contrast to other infections.

Since the beginning of the pandemic, the majority of COVID-19 testing efforts have been focused toward qualitative detection of viral RNA or antigen, or measure of antibody against SARS-CoV-2 (neutralizing or binding). A simple and rapid 'detected' or 'not detected' result is all that is needed to quickly identify and take appropriate actions like public health mitigation or decisions on the level of personal protective equipment use during a procedure. However, as our understanding of COVID-19 deepens and our experience with this virus extends beyond acute infection and public health mitigation, there is a growing need to understand viral burden and viral load trending for both research purposes and patient management.

2.1. Need for SARS-CoV-2 quantification and viral load trending

Viral nucleic acid quantification is an important and critical factor in various types of patient management settings. Quantitative viral load may inform clinicians whether the patient is responding to viral suppression therapy, detection is from a new infection (or reactivation) vs. prolonged shedding of an old infection, or creeping viral load points to a potential treatment failure in patients undergoing long-term therapy. The utility of viral nucleic acid quantification is well exemplified in post-transplant monitoring, where low-level viremia persists due to impaired immune clearance from immune suppressive therapy. Various guidelines and respective institutional policies have employed actionable viral load levels to initiate, stop, or change therapy in Cytomegalovirus (CMV) and BK virus (BKV) monitoring [18–20].

While evidence is still emerging on the role of viral load and COVID-19 prognosis and transmission, knowing SARS-CoV-2 viral quantitation may be clinically and epidemiologically useful in some situations. Various studies have shown that prolonged SARS-CoV-2 RNA shedding can occur in both immunocompetent patients that have recovered from COVID-19 [21,22] and those who are on suppressive therapy [23,24]. Similar to BKV, Epstein-Barr Virus, or CMV viral shedding in severely immune compromised or suppressed patients, including transplant patients, seem to experience prolonged SARS-CoV-2 RNA shedding months after initial COVID-19 diagnosis [24–26].

In a recent study, Aydillo et al. looked into the viability and RNA shedding in oncology patients [24]. The study enrolled 20 patients, 15 of whom received active chemotherapy. In these patients, a longitudinal respiratory specimen test series showed that SARS-CoV-2 RNA was detectable for up to 78 days from onset of symptoms. Viable virus was initially isolated in 10 out of 14 patients with a nasopharyngeal swab collection. Five of these patients had prolonged shedding ranging from 1 week to 2 months. Of these five patients, viable virus was isolated in three patients beyond 20 days of onset of symptoms. Two of these three patients had severe COVID-19 and received investigational treatments. Understanding viral load trends and kinetics, and not simply just qualitative detection, may provide better guidance in managing unique patient populations.

In a metadata analysis, Cevik et al. looked at 79 studies (5,340 individuals) that reported duration of SARS-CoV-2 shedding in various specimen types [27]. The analysis included 43 studies reporting on the upper respiratory tract with a mean duration of 17.0 days, 7 studies reporting on the lower respiratory tract with a mean duration of 14.6 days, and 13 studies reporting on stool with a mean duration of 17.2 days. The authors identified 11 studies that attempted virus isolation, of which 8 successfully isolated virus in respiratory specimen within 1 week of onset of symptoms, and no virus was isolated beyond 9 days. In studies that correlated with viral load, viral isolation was not successful from specimen with estimated low viral quantitation. It's important to note that the metadata analysis did not stratify patient risk groups, like oncology or transplant patients undergoing suppressive therapy. The fact that most study participants are immunocompetent could

explain the shorter window of viable virus compared to studies focused on immune suppressed individuals.

Even in patients who do not experience prolonged SARS-CoV-2 shedding, knowing the viral load rather than just a qualitative 'detected' vs. 'not detected' could provide guidance to infection prevention specialists when considering initiation or discharge of airborne precaution. While additional studies on transmissibility is needed, a number of studies have shown lack of infectivity in viral culture from specimen with low-level SARS-CoV-2 viral load [27–29]. Therefore, depending on the clinical scenario, hospital ICU census, availability of personal protective equipment, and stepdown protocols, information on viral load trending could help make better informed decisions when needed. Additionally, as targeted antiviral treatments for COVID-19 become available, quantification of SARS-CoV-2 could play an important role in assessing treatment response and management decisions in immunocompetent patients.

2.2. Current approaches to SARS-CoV-2 quantification

Various approaches are currently used to estimate SARS-CoV-2 viral load. Although there are no FDA approved or EUA assay for the quantification of SARS-CoV-2, some laboratories are reporting cycle threshold (Ct) values from qualitative PCR assays as surrogate estimation of viral load for both research purposes, and also in clinical settings for clinical and infection control decision making. Recently, the FDA clarified that reporting Ct values from qualitative SARS-CoV-2 PCR assays with EUA status is allowed if the platform provides such information [30]. While the FDA does provide limitations and caution statements on how Ct values should be interpreted, it reasons that Ct values could be used as a measure of estimation of viral load, and could provide guidance in evaluating disease burden and transmission. However, given that quantitative viral load is typically reported as a defined quantity or unitage, like IU/mL or Log copies/mL, and not as a Ct value, reporting Ct value in clinical setting has led to some confusion and inconsistency.

Quantification of virus is generally performed by constructing a standard curve using dilution series of a qualified reference material on a PCR platform and evaluating linearity against corresponding Ct values. Gold standard reference material is usually derived from the World Health Organization (WHO) international standard, when available. Clinical samples are then tested on the validated PCR instrument along with the appropriate quality control samples. The PCR instrument generates a Ct value, and the quantitation is derived from the standard curve. Thus, rather than interpreting a Ct value, clinicians get quantified viral loads, which can be compared across different platforms and methodologies. Thus, a result from one institution can be interpreted at another institution.

While Ct values from qualitative EUA assays can provide some insights into disease burden and potentially help navigate isolation and quarantine discontinuation, there are major limitations to consider in reporting Ct values. First, EUA assays from different manufacturers have different Ct value ranges. An assay from one platform may have a reportable Ct value range of 10 to 40 while another

platform may have a range of 10 to 35. Thus, a Ct value of 35 will have different interpretation depending on what platform the specimen was tested on. Given the large testing volume and the need to build redundancy around reagent shortages, many clinical labs are running multiple platforms, which have different detection ranges and Ct reporting. Secondly, different primer/probe designs will have different PCR efficiency and binding properties. Not only will this result in differences in Ct values, but also provide inconsistent interpretation when trending Ct values. A difference of three cycles from a prior test result performed may hold different significance with different platforms, and certainly not interchangeable. Lastly, and probably the most important point, is that a respiratory specimen, especially the commonly collected nasal and nasopharyngeal swabs, are not homogeneous specimen types. Unlike serum or plasma, respiratory specimen collection is largely dependent on sampling techniques. Depending on the rigor, depth, and contact time of the swab during collection, varying amounts of viral RNA and host cellular materials may be collected. While collection technique is important for qualitative testing, it is absolutely critical for quantitative testing.

Some of the limitations discussed in reporting Ct values are exemplified by recent data reported by the College of American Pathologists (CAP), an organization that accredits clinical laboratory under the Clinical Laboratory Improvement Act (CLIA). As part of accreditation, CAP requires laboratories to perform proficiency testing for each of the analyte/test the laboratory offers as a means of evaluating laboratory performance. For laboratories subscribing to the CAP proficiency testing program, CAP sends unknown samples to participating laboratories and grade their results. For SARS-CoV-2 qualitative PCR testing, CAP recently published data from a proficiency testing survey of over 700 participating laboratories [31]. As with any proficiency testing survey, the same batch of test material was sent. Participating laboratories were asked to report back Ct values in addition to qualitative results. CAP received data on eight different EUA assays with various assay designs for detection, two targets with separate probes, two targets with a combined probe, and a single target with a single probe. The median Ct value range among the different platform was 14 cycles. Interestingly, differences in Ct value tested in different laboratories using the same platform were as high as 3 cycles. To date, this is the largest analytical performance evaluation of SARS-CoV-2 qualitative PCR assay in a clinical laboratory setting, and the results point to major pitfalls in Ct value commutability.

Some studies in research settings have reported quantitative viral load in units like copies/mL, instead of Ct values. One study reported SARS-CoV-2 viral load as \log_{10} copies/mL by deriving a standard curve using the 2019-nCoV/USA-WA1/2020 strain [27], while another study also reported viral load as \log_{10} copies/mL but deriving a standard curve using constructed plasmid with synthetic viral transcripts [32]. While converting Ct values to a quantitative value derived from a standard curve greatly helps with many of the challenges around reporting raw Ct values, consistency of sample collection and adoption of the recently established WHO standard reference material still remain as major drawbacks, not to

mention commutability and use of consistent unitage for reporting.

It is important to note that there was no WHO international standard for SARS-CoV-2 until recently [33]. The Expert Committee on Biological Standardization (ECBS) approved the first WHO international standard for SARS-CoV-2 at the December 2020 meeting [34]. It is now available through the National Institute for Biological Standards and Control (NIBSC). Whether governments and test developers around the world adopt the new WHO international standard remains to be seen.

In the US, the FDA is gathering comparative data using a reference panel to assess cross-reactivity with another coronavirus and compare analytical sensitivity across EUA assays [35]. The reference panel, which consists of heat-inactivated SARS-CoV-2 strain 2019-nCoV/USA-WA1/2020, was distributed to close to 200 test developers. Following a specific FDA protocol, a limit of detection (LoD) can be derived. The FDA undertook this initiative to evaluate the LoD of EUA assays using the same material in a standardized approach given the fact that a plethora of standard materials were used to develop these assays, resulting in difficulties comparing and contrasting LoD's across EUA assays. As of this writing, 117 participating test developers reported results, and the LoD ranged from 180 to 600,000 NAAT Detectable Units/mL (NDU/mL). NDU is defined as a single detectable unit that is sufficient to provide a positive test result [36]. While the purpose of this data gathering was to compare and contrast LoD across EUA assays, a similar initiative with serial dilutions of a standard material to build standard curves could provide insights into quantitative capabilities of these tests.

2.3. Future perspective on SARS-CoV-2 quantification

Qualitative results alone are sufficient to diagnose COVID-19 cases. However, in unique patient populations, information on viral load trends can provide important insights that can help with further management and better informed infection control decisions. Additionally, as specific antiviral treatments for COVID-19 become available, quantification of SARS-CoV-2 will be needed to assess treatment response and monitoring. The current use of Ct value as a surrogate to viral load estimation has many limitations, and even quantitative viral load reporting has its challenges. A path forward for standardized SARS-CoV-2 quantification is needed.

First, the most urgent need with SARS-CoV-2 quantification is the harmonization of a reference standard for test development and calibration within and among platforms. While the WHO international standard has recently become available consisting of acid-heat inactivated England/02/2020 strain of SARS-CoV-2 [33], the vast majority of tests were already developed and verified using other reference materials. In the US, the most widely used SARS-CoV-2 reference material for test development is the 2019-nCoV/USA-WA1/2020 strain, a viral isolate obtained from a patient who returned to Washington state after traveling to an affected region of China in January 2020. Different preparations of the product, like extracted RNA, cell culture lysate, and heat-inactivated lysates, are available. Importantly, the 2019-nCoV/USA-WA1/2020

strain preparations are available as products from a variety of reference material manufacturers. This has resulted in variation in propagation and passage among different manufacturers as evidenced by different cell line used for viral culture to manufacture the products, which could impact the quality and commutability of the material. Therefore, a concerted effort is needed to standardize the manufacturing of reference materials, or move toward wider adoption of the WHO international standard. Ideally, the use of a single material would be ideal. However, similar to reagent and consumable shortages experienced during this pandemic, having redundancy in reference standards that is commutable could be the best approach.

Second, whether it is the 2019-nCoV/USA-WA1/2020 strain or the recently established WHO international standard England/02/2020 strain, the condition of SARS-CoV-2 RNA derived from cell culture may not reflect viral RNA in vivo. Therefore, further research into the species of RNA and their relative abundance found in clinical samples, including RNA in viruses, circulating RNA, and RNA intermediates and subgenomic RNA, and the extent of fragmentation and impact on detection and quantification, is needed. In other viruses, various studies have shown lack of commutability of WHO international standards, resulting in large differences in quantification [37,38]. This includes issues around fragmentation and amplicon size. In the ECBS report, the committee acknowledged that commutability of the WHO international standard for SARS-CoV-2 RNA has not been thoroughly evaluated due to time constraints and the level of risk involved in undertaking such studies within a reasonable timeframe [33]. Given that differences in assay design can result in differences in quantitation even when using the same reference material, extensive evaluation to assess commutability of the reference standard is needed.

Lastly, variability in sample collection needs to be addressed and mitigated. While education and training can streamline collection techniques, sample collection is inherently variable for specimens like nasal and nasopharyngeal swabbing. Therefore, rather than relying on the hopes of consistent technique of sample collectors, an assessment of the sample integrity and subsequent normalization may be a better approach. One option is to normalize the viral quantitation with host cell quantitation as measured with housekeeping genes and report viral load as NAAT detectable unit (NDU) per host cell. Some of the SARS-CoV-2 RNA detection assays include host nucleic acid detection like RNase P. While there are limitations of using RNase P in its current formulation, like detection of both DNA and RNA in the same reaction, other housekeeping genes or a different primer/probe design specifically targeting host DNA to derive an estimation of host cellular material would be needed. Thus, rather than reporting per mL of sample, quantification would be normalized based on how much host epithelial cells are collected, with the assumption that a higher number of epithelial cells may equate to a better collection. Establishing standard curve using a reference material in IU per mL or copies per mL, then converting that into an NDU per host cell may require some background calculations. For other sample types like bronchoalveolar lavage fluid (BAL) or sputum, where sample

collection technique can be considered more consistent than swabbing, NDU per mL may be more appropriate. One point to note, kinetics of viral shedding has been shown to vary in different anatomic sites, especially in relation to anatomic sites of viral entry [39,40]. Therefore, quantification should be limited to serial collection of a single or specific sites for consistent result interpretation. To account for shedding at different anatomic sites, perhaps a stratification approach based on patient risk factors, and sampling at multiple sites when feasible, could be devised into an algorithm.

3. Conclusion

As our experience with COVID-19 continues to evolve, the need for SARS-CoV-2 quantification is growing in certain situations. In patients with prolonged RNA shedding or in assessing infection control measures for patients that are already qualitatively positive for COVID-19, evaluating SARS-CoV-2 viral load trends could provide useful insights. Whether a single viral load test result can provide meaningful or actionable information without viral load trend data remains to be seen. In developing a quantitative SARS-CoV-2 test, there are a number of limitations to consider, including imperfect specimen collection, imperfect reference materials, and imperfect knowledge of the virus species and state *in vivo* that can impact quantification. As the field awaits solutions to these limitations, it is important to keep a perspective on minimizing risks and maximizing benefits in developing an assay with these limitations. Perhaps, the risk is minimized by narrowing the indication for testing or restricting the testing population. In the alternative, reporting quantitation based on a standard curve, regardless of the reference material, may be more beneficial than simply reporting Ct values.

4. Expert opinion

The speed at which diagnostic tests were developed, deployed, and implemented during this pandemic has been unprecedented. As test manufacturers, government grant agencies, regulatory bodies, testing laboratories, clinicians, investors, and the public at-large conduct postmortem analyses, one thing will become clear: the *in vitro* diagnostic space will never be the same. Prior to the pandemic, most people did not know what a nucleic acid amplification test (NAAT) was, or that an antigen test is different from an antibody test. The concept of pretest probability and false positive or negative test was foreign to most people, no less that medical laboratory professionals perform these diagnostic tests and provide result interpretation. Now, these terms and concepts have become common parlance, and for better or worse, we live in a world with a greater and expanded general knowledge of diagnostic testing.

Coupled with this crash course in diagnostic testing, the use of emerging technology and fast-tracking some of the previous regulatory huddles have demonstrated that we can think outside the box and it may be time to let go of some of the antiquated dogma that has strapped down the *in vitro* diagnostic space in the past. Incubators and government-

sponsored programs have pushed diagnostic test development to new levels of collaboration and innovation. This shift has allowed the expansion of home-collection, self-collection, and at-home testing, not to mention smartphone and online-based result reporting and tracking, to name a few. It has opened avenues for over-the-counter (OTC) and direct-to-consumer (DTC) respiratory viral antigen and antibody testing, and NAAT with newer platform designs.

To that effect, while it may have been dismissed in the past, quantification of respiratory viruses from swab collections may find a place to root. However, caution should not be thrown to the wind. In the midst of this exciting and revolutionary times in the *in vitro* diagnostic space, manufacturers and regulatory agencies, and clinical and laboratory professionals must all work as stewards to ensure that innovation and progress is rooted in evidence. This includes robust collection devices and methods that are optimal for quantification, reliable commutable reference material, and well-designed clinical studies evaluating viral load trends. Ensuring public confidence by gaining and maintaining public trust through evidence is pivotal, especially in the current world of social media and 'viral' spread of misinformation.

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