REVIEW

Considerations for the selection of tests for SARS-CoV-2 molecular diagnostics

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Abstract
During the course of 2020, the outbreak of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) spread rapidly across the world. Clinical diagnostic testing for SARS-Cov-2 infection has relied on the real-time Reverse Transcriptase Polymerase Chain Reaction and is considered the gold standard assay. Commercial vendors and laboratories quickly mobilised to develop diagnostic tests to detect the novel coronavirus, which was fundamentally important in the pandemic response. These SARS-Cov-2 assays were developed in line with the Food Drug Administration-Emergency Use Authorization guidance. Although new tests are continuously being developed, information about SARS-CoV-2 diagnostic molecular test accuracy has been limited and at times controversial. Therefore, the analytical and clinical performance of SARS-CoV-2 test kits should be carefully considered by the appropriate regulatory authorities and evaluated by independent laboratory validation. This would provide improved end-user confidence in selecting the most reliable and accurate diagnostic test. Moreover, it is unclear whether some of these rapidly developed tests have been subjected to rigorous quality control and assurance required under good manufacturing practice. Variable target gene regions selected for currently available tests, potential mutation in target gene regions, non-standardized pre-analytic phase, a lack of manufacturer independent validation data all create difficulties in selecting tests appropriate for different countries and laboratories. Here we provide information on test criteria which are important in the assessment and selection of SARS-CoV-2 molecular diagnostic tests and outline the potential issues associated with a proportion of the tests on the market.

Keywords SARS-CoV-2 · COVID-19 · Real-time RT-PCR · Diagnostic performance · Test selection

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Introduction

The World Health Organization (WHO) released a statement officially confirming cases of pneumonia with unknown origin in Wuhan City, Hubei Province on 31 December 2019 [1]. On 9 January 2020, a novel coronavirus was announced as the causative agent by the Chinese authorities and later officially named Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-Cov-2) and responsible for the disease known as COVID-19 [2]. The number of confirmed cases rapidly increased and spread to other countries as the pandemic developed across the globe [3].

The COVID-19 pandemic continues to spread rapidly manifesting as second and third waves of increased transmission with concerns of important viral transmission by asymptomatic or moderately symptomatic patients [4]. During pandemics, rapid highly sensitive diagnostic tests play an essential role in epidemiological control and clinical management by identifying infected individuals and ensuring disease management to prevent the spread of the infectious agent and save lives [5].

The WHO stated that testing was a critical factor in controlling the spread of SARS-CoV-2 [6] (https://apps.who.int/iris/handle/10665/331509) and subsequently re-emphasized the call to include retesting due to the low accuracy of some of the available tests and associated pre-analytical issues [7–9]. Polymerase Chain Reaction (PCR) based methods are the gold standard in virus detection and are the assay of choice for the diagnosis of SARS-CoV-2 [10]. On 13 January 2020 the WHO released SARS-CoV-2 diagnostic testing guidance and the first real-time Reverse-Transcriptase PCR (RT-PCR) test was published by Corman et al. in January 2020 [11]. Further, the FDA released guidance on Emergency Use Authorisation (EUA) procedures for laboratories on 29 February 2020. The EUA supports emergency preparedness and response and fosters the development and availability of medical products for use in emergencies [12]. The FDA also updates the list of tests that have received EUA approval on its website [13].

During the development of a new diagnostic test, its performance should be compared using another device, a recognized reference method or clinical criteria for diagnosis. Also, validation studies examining the clinical performance of test are required before approval by the FDA [14]. However, understanding of FDA-EUA approval has been confusing as many clinical laboratories have mostly only experienced working with over-detailed FDA-IVD kits for which approval is highly complex and stringent [15]. Compared to FDA-IVD approval, the process for FDA-EUA approval is less stringent and mainly focused on analytical performance criteria. As the COVID-19 pandemic unfolded, the FDA-EUA recommended in the template for FDA-EUA approval of SARS-CoV-2 molecular-based tests the inclusion of Limit of Detection (LoD) as well as analytical sensitivity, cross-reactivity for analytical specificity and clinical evaluation for performance evaluation [16]. However diagnostic sensitivity, diagnostic specificity, positive predictive value and negative predictive value are among the basic and essential performance criteria for clinical diagnostic tests and have largely not been applied for SARS-CoV-2 molecular-based tests [17, 18]. Information on SARS-CoV-2 kits that have received FDA-EUA approval up to 1st September 2020 is contained in Supplementary Table 1. The instruction for use (IFU) supplied with these kits was used to extract information on analytical performance, target gene, sample volume, sample type and turnaround time of each test kit [13].

In several published studies, it has been suggested that some issues with SARS-CoV-2 detection were associated with pre-analytical and analytical factors. These included the lack of standardization of specimen type, the time of sampling, sample storage conditions, contamination, the use of insufficiently validated and verified assays, low viral load related to disease phase and recombination or mutation of viral genes [15, 19]. Here, in this review we examine the basic test features which are important in the selection of SARS-CoV-2 molecular diagnostic tests and discuss the existing problems of current tests that affect test selection.

Molecular diagnostic test selection criteria

The WHO's guide to aid selection of diagnostic tests describes the ASSURED (Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free and Deliverable to end-users) criteria which is considered the benchmark for determining if a diagnostic test is fit for purpose depending on need [20]. For SARS-CoV-2 the first aspect of this practical guide is defining the test purpose and includes determining if (i) the required test is qualitative or quantitative, (ii) it is a point-of-care (POC) test or a central laboratory test, (iii) the test will be performed manually or by automated instrumentation and (iv) it requires specialist skills and who will perform the work (laboratory technician or healthcare worker) [21]. Although a number of different assays have been developed for the detection of SARS-CoV-2, the quantitative RT-PCR assay is considered the gold standard. The RT-PCR reaction can be performed in one step or two steps. The one-step RT-PCR performs both reverse transcription and amplification in the same tube. Since technician-based errors, sample mismatching, aliquoting, pipetting errors are lower; the potential risk of cross-contamination is lower. It is also cheaper and easier to set up and is ideal for high throughput applications. Further,

gene specific primers are used to generate the cDNA leaving no cDNA template for the amplification of other gene targets. In the two-step method, the cDNA synthesis and RT-PCR reactions are performed in separate tubes. Additional pipetting steps increase the risk of pipetting errors and cross-contamination. However, two-step RT-PCR is more sensitive than one-step RT-PCR and it is possible to perform reverse transcription of other gene regions and the cDNA can be stored for later additional use. The preferred technique for detecting SARS-CoV-2 is one-step RT-PCR as it is quicker and easily integrated into liquid handling robotic platforms and automated systems [22–24]. Isothermal nucleic acid amplification is another technique used to detect SARS-CoV-2 and is especially useful for POC tests as it does not require a thermal cycler. Amplification occurs only at one temperature under isothermal conditions and can be performed in a single tube, without the need for sophisticated instrumentation. Droplet digital PCR (ddPCR), gene chip and loop-mediated isothermal amplification are the other nucleic acid based detection methods that have been used for the detection of SARS-Cov2. Nevertheless, the RT-PCR remains the predominant method for SARS-CoV-2 detection [11, 25, 26].

The second part of the ASSURED guide focuses on reviewing market available tests and includes appraising the workflow, technical characteristics, practicability, and test kit applicability. Basic information such as manufacturer details, the kit catalogue number, storage conditions of the kit, shelf-life stability (temperature, humidity, pressure), in-use stability (opened pack stability, opened vial stability and onboard stability if it is used with an instrument), and shipping stability are required. In addition, other information including appropriate specimen type (such as blood, oropharyngeal swab, nasopharyngeal swab, sputum or faeces), the required sample volume, control reagents, total turnaround time, RNA extraction and additional equipment requirement is required. Finally, instrument size, cost, education needs of the users and frequency of education, installation and maintenance requirements should be determined [21, 27].

Practicability dictates whether a new assay can be easily combined with other assays currently used in the laboratory and can be performed under the same conditions. Hence, determining if a new assay will incur additional cost and impact on the workforce is needed [28]. Regarding the technical and workflow characteristics of test kits, it is important to choose those with ready-to-use reagents, a small number of reaction tubes, and a limited number of pipetting steps to reduce the risk of contamination which is an important potential cause of false positive results [29]. This is especially critical during a pandemic when high throughput (number of samples in a run) and short turnaround time are demanded. Long turnaround times can result in the backlogs

and increase in workload and number of patients in pandemic outpatient clinics requiring increased isolation periods [29]. Current sample production capacity, the total time required to perform the test and the number of tests each device can perform on an hourly and daily basis in theory and in practice should be taken into account [30].

The matrices that test kits can work with and the limit of detection values of different matrices are reported in the IFU documentation of the kits [16]. A test kit compatible with the sample type to be analysed needs to be selected. Particular consideration should also be given in regard to matrix interfering substances and inhibitors which may cause the assay to fail [31]. Mucin, blood contamination, nasal sprays, drops, corticosteroids and gels, throat lozenges, oral anaesthetic and analgesic drugs or sprays, anti-viral and antibacterial drugs, haemoglobin, conjugated and unconjugated bilirubin, proteins in circulation, lipids, antibodies and rheumatoid factors can all interfere with the PCR reaction. Evaluation of potential interfering agents is therefore highly pertinent for new techniques [30].

The third part of the ASSURED criteria focuses on the analysis of the approvals granted by international and national organizations [21]. Manufacturers can obtain approval for SARS-CoV-2 molecular diagnostic kits from regulatory authorities such as CE-IVD, FDA-EUA, Australian Therapeutic Goods Administration (TGA), Singapore Health & Safety/Sciences Authority (HSA), Korea Ministry of Food & Drug Safety EUA (Korea-MFDS-EUA), Health Canada, China National Product Administration (NMPA)-EUA [32]. The regulatory approvals of the kits are shown in Supplementary table 1.

The final part of the guide involves the review of test performance under optimum conditions and clinical laboratory conditions as well as monitoring of the test during routine use, including quality control and assurance. The diagnostic accuracy of the test should be checked in peer reviewed publications and data from the manufacturer [21]. Importantly, data provided by manufacturers should be verified by post-market assessment [33] (https://asm.org/Articles/2020/April/False-Negatives-and-Reinfections-the-Challenges-of).

Challenges in selecting Sars-Cov-2 molecular diagnostic tests

Diagnostic performance challenges of SARS-CoV-2 diagnostic molecular tests

Analytical sensitivity and specificity should not be confused with diagnostic sensitivity and specificity as each has different meanings [34]. High analytical sensitivity does not necessarily equate to a high diagnostic sensitivity, and similarly, a test with high analytical specificity does not warrant that

the test has high diagnostic specificity. Analytical sensitivity is the smallest amount of a substance that can be detected by an assay and is known as the limit of detection (LoD) [16].

It is important to demonstrate a low LoD, which indicates greater analytical sensitivity and is likely the most common data provided by assay developers [18]. The SARS-CoV-2 kits granted FDA-EUA approval and the LoD values of the target genes of the tests are shown in Supplementary Table 1.

According to published studies on RT-PCR based assays for SARS-CoV-2 the reported diagnostic sensitivity ranged from 59 to 71% [35, 36]. It is known that diagnostic sensitivity is especially important in determining false negativity [17, 34]. During the COVID-19 pandemic, an enormous volume of tests have been performed and the number of infected individuals that test negative (i.e. false negative rate) constitutes a significant risk for pandemic control. There are a number of factors that may contribute to false negative results such as low viral load prior to onset of symptoms, insufficient sampling, unsuitable transport/storage conditions and mutation in the gene target site. The impact of false negative test results may create unnecessary confidence and increase the spread of the disease [37].

False positivity and cross-reactivity are also important issues that currently have not been well evaluated [38]. In low prevalence settings, the false positive rate has been found to be proportionally higher than in the high prevalence settings. However, in the high prevalence setting, individuals with a false positive result also have a major risk of viral exposure if isolated with patients with active COVID-19 infection. False positive results may cause delay of surgeries, workforce loss and unnecessary treatment and isolation [39]. In the real word setting it can be difficult to determine whether the source of false positivity is due to sample cross contamination, contamination during sampling from surface or gloves, cross reactions with other viruses or intrinsic analytical issues associated with some kits [38].

Non-standardized pre-analytical factors

There are numerous pre-analytic, analytic and post-analytic factors that affect performance, accuracy and repeatability of a diagnostic test [40, 41]. In some studies, it was mentioned that the high false negativity and false positivity rates of SARS-CoV-2 RT-PCR kits were mainly related to the pre-analytical phase, in particular sampling location, sampling time, transport and storage conditions [19, 40, 42, 43].

It has been found information on sample type and timing aimed at improving test accuracy change day by day. In the Korean guide, both nasopharyngeal and oropharyngeal sampling was recommended, while the United States Center for Disease Control and Prevention (US-CDC) recently updated their sampling procedure [44, 45]. It was established that sputum is the most sensitive sample for SARS-CoV-2 nucleic acid detection, with nasopharyngeal swabs the next most sensitive [46]. However, sputum induction produces an increased risk of aerosol transmission. There are also studies on the diagnostic value of saliva and faeces samples, with saliva being an easily obtained sample, but is not the preferred sample specimen [47, 48]. Due to the rapid release of updates, sample type, matrix effect and applicability features of kits should be taken into consideration [28]. The ability of kits to work with various matrices will make it easier to adapt to changing sampling procedures [44, 49].

Another crucial issue is that SARS-CoV-2 is an RNA virus and since the structure of RNA is much more sensitive than DNA to environmental factors, the integrity of RNA viruses can be more affected by transport and storage conditions [50, 51]. Swab variety, different viral transport media and PCR inhibitors can affect PCR-based viral detection [9, 46]. The US-CDC reported that swab samples should only be collected with a synthetic-tipped swab and the swab should be used with an aluminium or plastic shaft. Cotton swabs are not recommended in the US-CDC guideline, while transport of cotton swabs in viral transport medium (VTM) is considered appropriate in the Korean guidelines [44, 52]. Recently it has been reported that samples collected in VTM reduce the sensitivity of POC COVID-19 tests and the US-CDC updated guidance also noted that for some POC tests the use of VTM is not advised [44, 53]. In addition one manufacturer added further information to their IFU; indicating swabs should be placed directly in the POC instrument for testing and specimens in VTM are not an appropriate sample type [54].

In the Chinese Center for Disease Control (China-CDC) guidelines for SARS-Cov-2 molecular assays, there is no detailed information about swab suitability for different specimen types, but it is stated swabs can be placed in different VTM such as isotonic saline solution, tissue culture solution, or phosphate buffer solution [49]. The US-CDC has also published a standardized VTM contents and protocol to enable laboratory VTM reagent preparation [55]. Further, some SARS-CoV-2 RT-PCR kits contain specific swabs and VTMs and these kits are not guaranteed to work when using different swab types and VTM [45, 49, 55, 56]. The specimen collection and transport materials that are supplied with any product should be evaluated during test selection. Additionally, the pre-analytic phase gains more importance for home sample collection kits. An effective self-sampling of patients, contamination of the swabs, and the use of nasal swabs in self collection kits instead of nasopharyngeal swabs used in healthcare settings can have an impact on disease control by adversely affecting test results and clinical diagnosis [57].

Target genes for molecular diagnostic SARS-CoV-2 tests

Coronaviruses of the *Coronaviridae* family are enveloped zoonotic RNA viruses. Mammals serve as an intermediate host and contribute to coronavirus genetic diversity by facilitating recombination and mutation [58, 59]. The SARS-CoV-2 has a 29,903-nucleotide long viral genome with genes encoding structural proteins: spike (S), envelope (E), transmembrane (M), helicase (Hel), and nucleocapsid (N). There are also species-specific genes necessary for viral replication. These are RNA dependent RNA polymerase (RdRp), hemagglutinin-esterase (HE) and open reading frames ORFla and ORF1b [45, 60]. The N, E, S, ORF and RdRp are the target viral genes that have been used for molecular diagnostic tests, with test kits having numerous different primer sets directed against these regions [45, 61, 62]. Several SARS-CoV-2 RT-PCR based detection protocols have also been published by the World Health Organization [62] and the target genes of current SARS-Cov-2 tests from different organisations/institutions are shown in Table 1.

It is necessary to evaluate the analytical performance of different kits to ensure the test results are correctly interpreted. In in vitro sensitivity studies, the most sensitive target gene regions were determined to be the E gene and RdRp gene [11]. Comparison of the analytical performance and sensitivities of SARS-CoV-2 RT-PCR assays by the China-CDC, Charité (Universitätsmedizin Berlin Institute of Virology, Germany), US-CDC and Hong Kong University (HKU) revealed different analytical sensitivities between these assays when testing samples with low viral loads [63, 64]. Additionally, many of the available kits target different genes and do not provide the primer sequences data. This complicates kit selection as well as validation and verification of the sensitivity of the primer sets [65].

SARS-Cov-2 mutation and viral evolution

Mutations occurring within the primer–probe binding sequences of target gene are another potentially crucial issue in SARS-CoV-2 transmission and re-infection [33]. The analysis of 7666 SARS-CoV-2 genomes revealed 198 repetitive mutations in the SARS-CoV-2 genome. Numerous repetitive mutations have been detected in the Orf1ab in region encoding Nsp6, Nsp11, Nsp13 and the gene region encoding the S protein [66]. In another study 2,492 SARS-CoV-2 genome sequences were analysed and 1407 mutations were detected, of which 337 were in structural regions, including 173, 30, 25 and 109 mutations in S, M, E and N genes respectively [67]. Coding and non-coding mutations, SARS-CoV-2 variants show that SARS-CoV-2 exerts ongoing evolution [66–70]. Viral evolution can potentially

cause a decrease in the sensitivity of SARS-CoV-2 assays due to primer or probe binding mismatch [71]. One study reviewed 992 SARS-CoV-2 sequences and 12 nucleotide mismatches in the primary-probe binding region of at least two virus sequences were detected during the early stages of the pandemic [63]. Therefore, mutations and global diversity in the SARS-CoV-2 genome sequence are a crucial point when considering appropriate test selection. Whole-genome sequencing (WGS) can overcome the mutation-based problems which can increase the false-negative rate in RT-PCR based assays. However, WGS is not practical, cost-effective and applicable to clinical laboratories as a primary detection assay [72]. Nevertheless, analysis of SARS-CoV-2 target gene mutations and dynamic sequence analysis for determination of the targeted gene in the tests to be developed plays a vital role in the success of innovative tests [66, 72].

Recently, the United Kingdom reported the emergence of the SARS-CoV-2 Alpha variant B.1.1.7, which is highly transmissible and spread rapidly to other countries. Similarly, the Beta variant (known as B.1.351), Gamma variant (B.1.1.28.1) and Delta variant (B.1.617.2) have since been found in countries worldwide [73]. The FDA has published a letter to healthcare providers warning that mutations in the virus genome target regions may create primary probe incompatibility and that genetic variants can increase false negative results. The FDA also noted that tests that rely on the detection of multiple regions of the genome may be less impacted by genetic variation in the SARS-CoV-2 genome than tests that rely solely on detection of only a single region [74]. Therefore, the ability of kits to detect multiple variants and any updated test performance of kits should be taken into consideration. Specific SARS-CoV-2 variant detection kits may also be needed to screen for cases that are clinically compatible, abut have a high risk of false negativity [74, 75].

Inadequate SARS-Cov-2 kit verification

During routine use, it is imperative to monitor and document test performance and user complaints to the regulatory authorities. In Supplementary Table 1, each approved EUA test has its own characteristics and limitations. The verification phase is important in objectively evaluating the accuracy of kits. For this reason, on 3 April 2020, the American Society of Microbiology published a protocol describing the verification process of commercial SARS-CoV-2 kits, which are used in clinical laboratories [15, 18, 21, 33]. In the first step of verification, the information reported by the manufacturer is verified. In the second step, accuracy and precision studies are performed [15]. Accuracy is evaluated by comparing patient results with another EUA approved kit. Precision includes repeatability and reproducibility, it provides information about the study within-run variability, variability between-days, between runs, between lots,

Table 1 SARS-CoV-2 molecular test gene target regions [11, 62]

Country	Organisation/Institute	Gene targets forward and reverse primer sequences (5'-3')
China	Chinese Center for Disease Control and Prevention	Target 1: ORF1ab CCDC-ORF1- F: 5'-CCCTGTGGGTTTTACACTTAA-3' CCDC-ORF1-R: 5'-ACGATTGTGCATCAGCTGA-3' Target 2: N CCDC-N-F: 5'-GGGGAACTTCTCCTGCTAGAAT-3' CCDC-N-R: 5'-CAGACATTTTGCTCTCAAGCTG-3'
Germany	Charitè	Target 1: RdRp RdRp_SARSr-F: 5'-GTGARATGGTCATGTGTGGCGG-3' RdRp_SARSr-R: 5'CARATGTTAAASACACTATTAGCATA-3' Target 2:E E_Sarbeco-F:ACAGGTACGTTAATAGTTAATAGCGT E_Sarbeco-R:ATATTGCAGCAGTACGCACACA
Hong Kong SAR	Hong Kong University	Target 1: ORF 1b-nsp14 HKU-ORF1-F: 5'-TGGGGYTTTACRGGTAACCT-3' HKU-ORF1-R: 5'-AACRCGCTTAACAAAGCACTC-3' Target 2: N HKU-N-F: 5'-TAATCAGACAAGGAACTGATTA-3' HKU-N-R: 5'-CGAAGGTGTGACTTCCATG-3'
Thailand	National Institute of Health	Target 1:N WH-NIC N-F:CGTTTGGTGGACCCTCAGAT WH-NIC N-R:CCCCACTGCGTTCTCCATT
USA	United States Centers for Disease Control and Prevention	Three targets in N gene Target 1: N1 2019-nCoV_N1-F: 5'-GAC CCC AAA ATC AGC GAA AT-3' 2019-nCoV_N1-R: 5'-TCT GGT TAC TGC CAG TTG AAT CTG-3' Target 2: N2 2019-nCoV_N2-F: 5'-TTA CAA ACA TTG GCC GCA AA-3' 2019-nCoV_N2-R: 5'-GCG CGA CAT TCC GAA GAA-3' Target 3: N3 2019-nCoV_N3-F: 5'-GGG AGC CTT GAA TAC ACC AAA A-3' 2019-nCoV_N3-R: 5'-TGT AGC ACG ATT GCA GCA TTG-3'
France	Pasteur Institute, Paris	Two targets in RdRp gene Target 1: RdRP/nCoV_IP2 nCoV_IP2-F: 5'-ATGAGCTTAGTCCTGTTG-3' nCoV_IP2-R: 5'-CTCCCTTTGTTGTGTTGT-3' Target 2:RdRP/nCoV_IP4 nCoV_IP4-F: 5'-GGTAACTGGTATGATTTCG-3' nCoV_IP4-R: 5'-CTGGTCAAGGTTAATATAGG-3'

RdRp rna-bound rna polymerase, *E* envelope, *N* nucleocapsid, *ORF* open reading frame, *F* Forward, *R* Reverse

between operators and instruments [76]. Quality control is another component of monitoring the performance of a test in routine use [21]. Internal controls are necessary for evaluation of specimen quality, as well as RNA isolation and amplification steps [9]. This involves the use of an extraction negative control, extraction positive control, no template control and positive template control. External quality control assurance is a program in which blind panels of verified positive and negative samples are periodically sent to participating laboratories from a single coordinating center. The results of each laboratory are compared to the results of other laboratories and/or to an assigned value. Comparative results from all peer groups using the same method and device are reported to all participating laboratories. External quality assessment (EQA) materials usually include internationally accepted standards [77–79] (https://

). Information about internal quality controls for the extraction and amplification steps as well as external quality control materials and supply conditions can be obtained from EUA kit manufacturers.

Discussion

The COVID-19 pandemic has been a significant issue for global health, society and economies. The chief executive and general manager of the WHO, Tedros Ghebreyesus emphasized that countries should give priority to diagnostic testing and isolation by saying: "You cannot fight a fire blindfolded" [80]. However, what is more important than providing a result in clinical laboratories is to give an accurate result [81]. Diagnostic errors are critical clinically and economically and the effects of diagnostic errors are compounding during outbreaks [82]. Unnecessary quarantining and treatment of a patient that has a false positive test result can cause significant impacts on individuals, as well as loss of workforce and time for healthcare workers and unnecessary cost. In addition, false negative test results of asymptomatic or mildly symptomatic patients create a risk to pandemic control [19]. Surgical mortality and pulmonary complications may also occur due to peri-operative infection in patients with false negative pre-operative screening results and also pose a risk for surgical crew [83]. Further, convalescent immune plasma therapy is a promising treatment for SARS-CoV-2 and the eligibility criteria for donors and patients is determined by RT-PCR testing [84]. Therefore, high false negativity of SARS-CoV-2 diagnostic tests can challenge pandemic management at multiple points [19].

Rapidly produced SARS-CoV-2 RT-PCR tests have been able to obtain FDA-EUA and is a new classification for clinical laboratories. Also, diagnostic performance problems of SARS-CoV-2 molecular test have increased questions about diagnostic molecular test selection in clinical laboratories. In one study, false negativity rates of two tests that approved FDA-EUA were 14.8% and 11% [85]. Feng et al. recommended chest computer tomography to detect the first-period change of COVID-19 when RT-PCR test results are negative [86]. This is not only an issue for false negative and false positive results, but also for conflicting results that vary from initial and subsequent confirmatory tests and may be due to pre-analytical (viral load) or analytical factors [19, 87]. Many studies associate false negative results with the pre-analytical phase. Different studies have shown that viral RNA is detected in different patterns depending on sample type and the time of sample collection and the disease phase [46]. It is important to carefully consider pre-analytical variables that will affect test clinical performance. However, in a comparison study in which the pre-analytical phase conditions were equal for all kits tested, seven commercial kits (which were granted FDA-EUA) were compared with an in-house kit and the diagnostic sensitivity values ranged from 62.5% to 81.2% [88].

The accurate diagnosis of patients infected with SARS-CoV-2 is crucial to controlling the spread of SARS-CoV-2. However, some RT-PCR based diagnostic assays do not meet proper clinical diagnostic performance standards. These tests are recommended to be performed by trained staff in central laboratories and may not be widely deployed in undeveloped countries with limited health care facilities, or in remote locations. Although the diagnosis of SARS-CoV-2 by RT-PCR is mostly laboratory-based, molecular-based POC tests may be an attractive alternative due to reduced transport problems and relatively shorter turnaround times. POC tests can be used in any location, such as hospitals, clinics, emergency departments or remote locations [89, 90]. In Supplementary Table 1, molecular POC assays that use isothermal nucleic acid amplification technology for the detection of SARS-CoV-2 is shown. However, in comparative diagnostic accuracy studies, the performance of POC tests was found to have significant limitations for the diagnosis of SARS-CoV-2 [85, 91, 92]. Although NGS based detection provides better results, it is not a cost-effective and practical method for routine clinical diagnosis [93]. Similarly, ddPCR is more sensitive than RT-PCR and allows the detection of lower viral loads, but has low throughput and requires more specialised technical settings and equipment, making it unsuitable for widespread use [24, 25].

Epidemiologically regional and common mutations in primer and probe target regions in the SARS-CoV-2 genome should be considered in kit selection. Although primers and probes are specifically designed against protected areas of the viral genome, mismatches between primers and probes and target sequences can occur and result in decreased test accuracy [71]. These primary probe mismatch mutations can also affect assay annealing temperatures and increase the risk of dimer and hairpin formation of primers [94]. RT-PCR approaches that target multiple genes may overcome mutation based errors. This would potentially increase the amount of information gained from a single test, improve diagnostic specificity and reduce reagent usage and cost, as well as workload in clinical laboratories [17, 95, 96]. Considering that SARS-CoV-2 is continuously evolving and accumulating mutations, access to genome sequencing data to assist primers and probe design is an important requirement for assay developers and is likely to decrease false negative rates. Improving the multiplex properties of nucleic acid tests and integrating these with serological tests may also improve test accuracy [17, 95]. Further, combined tests may enable better differentiation of false negatives in the early and late phases of infection [19, 97].

The clinical performance of commercial kits should be evaluated objectively and publicly shared by the authoritative institutions. Rapidly produced tests do not go through the verification phase and clinical trials have been inadequate [37]. In order to provide users with more accurate diagnostic kits, several organizations have invited assay developers to evaluate their test products independently [27, 98]. In order to compare the kits, the LoD values obtained using standardized reference material are shown in Supplementary Table 1 [99]. The Foundation for Innovative New Diagnositics (FIND) is an organization that is working with the WHO and in partnership with the University Hospital of Geneva (HUG) to evaluate assays to verify molecular test kits detection limit, and clinical performance data reported by vendors. The evaluated commercial test data is now being published on the FIND website [98]. While diagnostic sensitivities ranging from 90 to 100% have been reported by FIND, the fact that tests perform at approximately 70% of diagnostic sensitivity in routine use emphasizes the importance of verification by end users [15, 35]. Monitoring and publishing the performance of kits will be a useful guide for users in selecting tests [33]. An important part of test performance monitoring is the quality control phase. External quality control is a critical process for the verification of molecular assay accuracy. It is recommended that an international external quality assessment is needed to assess the quality of assays used [38]. Procedures for internal quality control and external quality control of molecular tests and the provision of control materials with kits is also critical in test selection [81, 95].

It is clear that pandemics affect the economies of all countries, and successive waves of infection magnify this problem. Choosing a cost-effective high performance test by considering the above parameters during kit selection will enhance the role and success of clinic laboratories in pandemic management.

Conclusion

Early and accurate diagnosis of SARS-CoV-2 is the first of a number interventions required for effective pandemic management. It is essential to increase the diagnostic sensitivity and specificity of SARS-CoV-2 tests to minimize the impact of the pandemic on the global health system. Therefore, laboratories and national authorities should consider types of RT-PCR tests (one-step vs. two-step), pre-analytical factors (types of samples, interfering substances, transport conditions), diagnostic and analytical accuracy (specificity, sensitivity), target genes analysed, mutation variants of the virus, quality control requirements in PCR test selection. Further, laboratories should verify kits under laboratory conditions and monitor performance data. Requesting updated diagnostic performance data from manufacturers as part of the test selection process will encourage companies to evaluate their commercial kits by independent authorities.

Declarations

Conflict of interest The authors declare no competing interests.

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