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COMMENTARY

The Path Forward for COVID-19 Diagnostics

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The sudden onset of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; COVID-19) pandemic created an initial vacuum for in vitro diagnostics (IVDs). With commercial assays now available, the research and development of COVID-19 IVDs should focus on improving reaction sensitivity, reducing reagent usage, and minimizing testing time. At the time of writing, 41 COVID-19 IVD assays have been granted emergency use authorization (EUA) by the US FDA, 37 of which are nucleic acid based [1]. Serological tests offer much shorter detection time than nucleic acid-based approaches; however, their antibody affinity and specificity require significant periods of validation. The predictability of polymerase chain reaction (PCR)-based assays make them more suitable during this stage of viral testing. Since lockdown of the general public has ended in the USA, and the screening of individuals for work resumption has begun, the volume of COVID-19 tests needed has increased drastically. Therefore, rapid, sensitive, and—most importantly—accurate IVD assays are needed to address the expanded global screening.

Point-of-care (POC) testing has great potential for widespread diagnosis of COVID-19 because of its rapidity, low cost, and easy distribution. Currently, the most common techniques involve reverse transcriptase PCR (RT-PCR), but many recent POC approaches involve using loop-mediated isothermal amplification (LAMP). LAMP is attractive because of its sensitivity and short run time and because it does not require a thermocycler [2]. Other nucleic acid-based tests, such as those involving digital droplet PCR and nanoparticle-based DNA amplification, are also being examined because of their superior limit of detection (LOD), sensitivity, and specificity. Antibody tests for immunoglobulin G and M also exist, using lateral flow assays for a POC approach [3]. Lab-on-a-chip (LOC) and microfluidic devices show particular promise. As LOC devices can be miniaturized and easily automated, they are an excellent platform for POC diagnostics, and this is already being realized in the literature [4–6]. Innovations such as 3D printing have the potential to revolutionize LOC technologies and increase accessibility [7].

The use of various biological fluids has been proposed to test for COVID-19. Perhaps the most prominent of these is saliva, which has been shown to contain SARS-CoV-2 RNA [8, 9]. SARS-CoV-2 has also been detected in blood [10, 11] and fecal samples [11, 12], whereas several studies have shown it is not detectable in urine [13, 14]. There is potential for diagnostic tests to use any of these fluids that contain SARS-CoV-2, and development of these tests could allow for cross-verification between assays to ensure patients are diagnosed correctly.

The reagent usage of COVID-19 testing should be optimized through reaction scaling and improved efficiency. First, a technical advantage of IVD is its scalability; reactions can take place in nanoliter volumes such as in droplet PCR. The mass of enzyme and oligonucleotides required scales down proportionately. While droplet PCR can reduce reagent usage, further reduction can be achieved through the use of more efficient enzymes. Taq polymerase, the most commonly utilized enzyme for PCR, is less efficient than mesophilic polymerase for DNA replication. The adoption of more efficient polymerases, such as mutants of Taq, and various archaeal polymerases could reduce reagent requirements. Lyophilized enzymes and oligonucleotides are widely used in POC testing. Improvements to the stability of lyophilized enzymes could help to maintain optimal activity when resuspended, thereby improving the efficiency of POC assays.

Rapid COVID-19 testing should be achieved without compromises in reagent conservation and detection specificity. Through optimization of the length of the amplified region, RT-PCR testing can be performed in under 30 min, closing the gap with serological assays. RT-PCR can also be run in under 10 min using specialized thermocyclers, fulfilling a need for on-site COVID-19 readout; the concentration

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of DNA polymerase in such "ultra-fast" reactions is up to ten times that of regular reactions. The large amount of enzyme needed to achieve reaction speed parity with immunoassays makes "ultra-fast" PCR less ideal for population-wide testing. Recently, an isothermal amplification-based COVID-19 assay was granted EUA; this RT-LAMP assay from Abbott Laboratory could produce a result in under 10 min [15]. Compared with PCR, the presence of multiple pair primers in LAMP increases the formation of unspecified side product and reduces the amplification efficiency of the target sequence in samples containing a low concentration of virus. The sensitivity and specificity of RT-LAMP assays should be evaluated against a full range of COVID-19 viral loads.

This artifact of LAMP introduces two other important considerations in diagnostic testing: viral load and LOD. Detection limit has been explored in some papers in the literature, with many commercial kits tending to have a 95% LOD around 3–5 copies per milliliter [16]. The type of bodily fluid being tested also needs to be considered, as the amount of virus present varies considerably. At the time of writing, some studies have been performed to aid understanding of viral load at different stages of infection; these studies have shown that viral copies start at 10^9 copies/mL in sputum and 10^7 copies/mL from throat swabs and steadily decrease for the next 2 weeks [17, 18]. However, more conclusive studies could be conducted to examine viral load in the context of the LOD required for diagnostics to be effective, especially with respect to multiple different bodily fluids. This is particularly important since viral loads are known to vary widely across individuals, ranging from well above LODs of diagnostic tests to near or below them [19].

A further important factor to consider is the evaluation of false-positive and false-negative results. False-negative results have a risk of admitting infected patients into public areas and spreading COVID-19 further, whereas false-positive results risk patients thinking they are immune when they are actually not. Data in the literature about false-negative diagnoses are sparse. Over five studies, Arevalo-Rodriguez et al. [20] noticed a false-negative rate of 8.5%, with false-negative rates ranging from 2 to 29% over these studies individually. These potentially very high rates of false negatives, if accurate, could be disastrous. In the short term, testing patients multiple times seems to be a reasonable path of action to prevent these false-negative results, and improving RT-PCR and serological assays is important in the long term [20, 21].

In addition, isothermal amplification techniques, such as LAMP, have been shown to produce higher rates of false negatives than PCR [22]. On the other hand, few data are available for false-positive incidence of COVID-19 diagnostics, likely because the consequences of false positives are lesser than those of false negatives. Nucleic acid-based and serological techniques are prone to yielding false-positive results, and the false-positive rate varies with the prevalence of COVID-19. With 1% of the population infected, the false-positive rate is 80.8%; with 90% infected, this rate is 0.5% [23]. As such, to minimize incorrect diagnoses, the sensitivity and specificity of diagnostic tests must be optimized.

RNA viruses, such as HIV-1, have been shown to contain common single nucleotide polymorphisms (SNPs) that confer resistance to certain antiretroviral drugs. Similar SNPs could be found in COVID-19. A number of drug candidates, such as ritonavir, are being trialed for the treatment of COVID-19 [24]; the screening for common COVID-19 SNPs that confer drug resistance should be a concurrent priority. Many techniques towards RNA mutation testing are available, the most sensitive of which being next-generation sequencing. However, given the scope of the pandemic, PCR-based molecular mutation testing might be more suitable. These PCR-based techniques are well-studied [25] but have yet to be adopted in a POC setting.

Autopsy remains a versatile technique for diagnosing causes of death and has potential to provide important insight into the proper diagnosis of COVID-19 and as a way to gather clinical data for overall morbidity rates [26]. It also has the potential to act as a secondary test to evaluate experimental diagnostic tests for living patients and as a standardized method to compare SARS-CoV-2 diagnoses with those of other diseases that are better understood (for example, influenza). However, postmortem examination carries the added risk of virus transmission for healthcare professionals, so all autopsies require extreme care. Thus, it seems that the best course of action with postmortem examination is to restrict autopsies to cases with high potential to gather new information pertinent to diagnosis [27].

Finally, diagnostic tests can be used to understand environmental contamination. Many studies have confirmed that objects frequently in human contact harbor SARS-CoV-2, with contamination being the highest in the first week of illness [28, 29]. However, few studies have discussed the threshold at which environmental contamination carries a high risk of infecting individuals. Improved diagnostic tests could improve our understanding of this subject and help reduce infection rates in places with a high density of patients with COVID-19.

The number of asymptomatic COVID-19 cases is rising, and the wide testing of high-risk asymptomatic individuals creates challenges. Improvements in testing time and sensitivity, as well as screening for drug resistance, should be priorities in the path forward for COVID-19 diagnostics.

Declarations

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Conflicts of interest We (TU, LZ, AT) have no conflicts of interest to declare.

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