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COMMENTARY

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Guidelines on newly identified limitations of diagnostic tools for COVID-19 and consequences

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Abstract

Coronavirus disease 2019 (COVID-19) caused by coronavirus has spread worldwide and has become the deadliest pandemic of the 21st century. Such rapid spread is predominantly attributed to the poor diagnosis and its asymptomatic transmission. In the absence of treatment regime, timely diagnosis is the best available remedy that can restrict its spread. An early diagnosis of COVID-19 is critical for determining the line of treatment and preventing long term complications in the infected subject. Unfortunately, available rapid antigen and antibody kits are known to be erroneous whereas reverse transcription polymerase chain reaction based tests are expensive, viral load dependent and at times inconclusive. In current scenario, the false-negative results imposed a major risk to the individual patient care and also to the efforts for containing the spread at the population level, where as false positives are traumatic for families and can lead to improper treatment resulting in severe complications. In this article, the limitations of available diagnostic procedures have been elaborated and plausible combination approach has been advised.

KEYWORDS

biochemical analysis, coronavirus, research and analysis methods, virus classification

1 | INTRODUCTION

Till date, coronavirus disease 2019 (COVID-19) has infected over 47.3 million subjects and caused 1.22 million deaths, and the count is increasing.[1] Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, is a highly contagious pathogen that triggers mild to severe symptoms ranging from loss of smell and taste to fever, myalgia and acute respiratory distress.[2] Several studies have also reported the occurrence of skin rashes, neuropathy, nephropathy and cardiomyopathy in addition to gastrointestinal, liver, ocular and olfactory dysfunctions upon infection, and the list of symptoms is growing.[2] COVID-19 can spread through presymptomatic, asymptomatic and symptomatic carriers.[3] Its reproduction number has been calculated to be in between of 2 and 3 which suggest that each primary infected subject generate about 2 to 3 secondary cases.[4] The efficacy of the FDA approved drug remdesivir (Veklury) has been already contested.[5] Unfortunately, the accurate diagnosis for COVID-19 is still not possible due to various reasons that have been described in next sections. The correct diagnosis is absolutely vital for ensuring the right treatment regime, differentiate COVID-19 from other infections and for determining its presence in the patient population that can further facilitate implementation of suitable health policies.[3,6,7] Also, the timely diagnosis can limit the overuse of precious antibiotics.[7] False-negative COVID-19 test can cause more harm than perceived before especially in the absence of treatment regime and vaccine resulting in its spread.[7] Subjects infected with

COVID-19 usually display symptoms and consequent higher viral loads within a week of infection in their respiratory tract.[6,8] The key to the management of COVID-19 is in the understanding of most traits that can form the basis of clinical suspicion so that cases falling under the purview of latter can be subjected to appropriate diagnostic tests. Unfortunately, parameters for clinical suspicion of COVID-19 are still not well-defined and continuously evolving.[9] The clinical outcome in patients with COVID-19 can depend on various factors, such as age and co-morbidities like asthma.[10] As of now, subjects with fever, loss of smell and taste, myalgia and respiratory symptoms without any known cause have been included in the purview of clinical suspicion.[11] Further, subjects with a travel history to a country or place where community transfer has been reported are at the risk of infection.[12] In most countries, primary contacts of confirmed COVID-19 positive case are being traced and subjected to suitable isolation and diagnosis.[13,14] Its airborne transmission has been confirmed and face masks have been realized as the key determinant factor capable of inhibiting the interhuman transmission and preventing spread in primary contacts.[15] Surely, in the absence of treatment or vaccine, understanding of the mode of spread and the availability of accurate diagnostic tools can facilitate better tuning of the current and future public health policies.

1.1 | Inaccuracies at the step of collection of sample and erroneous time point

In this section, the caveats and source of variability at sample collection steps have been elaborated. For reverse transcription polymerase chain reaction (RT-PCR) based assays and antigen based rapid tests, a nasopharynx (NP) and an oropharynx (OP) swab are collected.[16] The incubation period that is the time between infection and onset of clinical symptoms varies from 5 days to 14 days in most patients. Typically, patients tested before the end of the incubation time period do not have enough viral load for detection and RT-PCR can potentially miss the infection.[17] Previously, it has been reported that 32% of OP and 63% of NP swabs could accurately determine the SARS-CoV-2 RNA.[18] Both NP and OP swabs taken collectively in one vial have shown higher accuracy for detection.[18] After collection, swabs placed in universal transport medium are transported to the microbiology laboratory under refrigerated conditions.[18] In antibody based assays, blood samples are collected and tested for the presence of antibodies against SARS-CoV-2.[19] Such antibody based point of care testing methods are highly error prone and mostly suitable for estimating the spread of virus in the population, which has been elaborated in other sections.

The expertize of the person collecting swab sample may also influence the accuracy of diagnosis. As per the centers for disease control and prevention guidelines, the swab should be inserted deep enough to cause flinching in the nasal cavity and then swab should be twirled three times for 10 s before pulling out.[20] Improper and insufficient swabbing may also result in false-negative. It is possible that in few cases at late stage of infection, sufficient virus load for detection can only be found in the lower respiratory tract and NP swab could become inadequate for viral load detection.[21] In such cases where subject is under the purview of clinical suspicion but still tested negative for RT-PCR, should be recommended for repeated testing of lower respiratory tract specimen like bronchoalveolar lavage (BAL) that has been reported to contain the high viral loads of COVID-19.[22] Several studies have confirmed the utility of BAL in the diagnosis of COVID-19 and BAL can be collected during intubation procedures.[22] Few patients with COVID-19 have also displayed high SARS-CoV2 RNA fecal material but it varies a lot therefore, its utility should be restricted only for studying the spread at the population level.[23] In summary, various factors such as the specimen site, appropriate protocol of specimen collection along with viral load at an anatomic location as a function of disease severity, and time of onset of symptoms (incubation period) must be considered before concluding the diagnosis.[6]

1.2 | Real-time reverse transcription polymerase chain reaction based molecular assay and its limitations

The COVID-19 RT-PCR test allows qualitative detection of SARS-CoV-2 nucleic acid in the specimens collected from upper (such as nasopharyngeal or oropharyngeal swabs and sputum) or lower respiratory tract (BAL) of suspected subjects. Unlike conventional PCR that amplifies dsDNA, RT-PCR comprises of reverse transcription of RNA (viral RNA) to dsDNA and its subsequent quantification by PCR.[24] RT-PCR machine computes cycle threshold (C_t) values that indicate cycle number required for the fluorescent signal to cross the threshold (exceeds background level).[16] The amplification curve should be sigmoidal which reflect proper amplification of the DNA. The confirmation of the sigmoidal curve is more important than anticipated as at times PCR plate contains unusually high level of fluorescent noise during early cycle of PCR which can lead to a false positive C_t value computation by the software. Here, Ct value shows the viral load in the processed sample. The higher Ct value corresponds to the lower viral load that is more PCR cycles are required for the detection of viral RNA whereas lower C_t value reflects higher viral load suggesting lower PCR cycle requirement.[25] As per the protocol, samples are subjected to 40 cycles of PCR along with a positive control gene. If Ct value is less than or equal to 37, sample is considered to be positive(+) whereas Ct value greater than 40 is considered to be negative(−). In case, C_t value ranges in between of 37 and 40, it is considered to be in the gray zone and requires more diagnosis before conclusion. As per FDA guidelines, gray zone samples are non-confirmatory and should be retested.[25] The first major limitation is the uncertainty of this test due to gray zone that is

subject is tested and still it is not clear if it is a positive case or a negative case. Second major drawback is the possibility of false negative results as a consequence of the degradation of viral RNA during shipping, handling or storage, improper swab collection, presence of RT-PCR inhibitors and lack of technical expertize.[26,27] The third limitation is the case of false positive, which is traumatic for subject and family, and risk complications due to other diseases with similar symptoms. False positives have been reported due to the RNA contamination, cross contamination between patient samples and in few cases nonfunctional PCR kit.[28,29] Subjects can test positive even after complete recovery due to the presence of nucleic acid which may take longer time to be cleared off from the system.[29] Fourth limitation is its high cost which has severe implications in developing and undeveloped countries where population pool is huge and people cannot afford such expensive testing. Several reports have contested the high accuracy of RT-PCR like in the case of referred report which concluded lower sensitivity of 60%–71%.[30] In the same report, authors described the utility of chest computed tomography scan imaging features in complementing RT-PCR that can reduce false negative results.[30] Importantly and unfortunately, RT-PCR has been reported to give positive test and detect viral RNA up to three months of recovery in several cases, therefore RT-PCR cannot be utilized for evaluating recovery upon discharge.[31] In fact, there is no test in practice that can conclusively confirm that subject is devoid of virus particles.

1.3 | Caveats of rapid antibody and antigen tests

In COVID-19 antibody test, the blood sample is examined for the detection of antibodies against SARS-CoV2.[32] Typically, subjects with COVID-19 infection develop antibodies in 1–3 weeks post symptoms.[32] Severe symptoms usually manifest higher titer of antibodies in comparison to the moderate symptoms or without symptoms in infected subjects.[33] The antibody response helps subjects in prognosis.[33] Antibodies donated by recovered patients are still being tested for efficacy and its potential in therapeutics.[4] As of now, antibody test is being used only for assessing the spread of the infection at the population or community level due to various reasons. First antibodies test cannot confirm infection for first few weeks because of lack of antibody and it will always be negative in initial days of infection.[34] Second, the antibody response varies from case to case and few COVID-19 positive patients never show any detectable antibody response or could not maintain the antibody levels for long, which may result in false negative test.[35] Third, different coronaviruses responsible for cold can also stimulate antibody response which can potentially give false positive test.[36] Usually, the immunoglobulin M (IgM) antibody titer starts rising after one week of infection and subsequently taken over by immunoglobulin G (IgG) antibody response after two weeks of infection.[37] IgG antibody titer can potentially last from 6 months to several years and their presence may suggest previous infection and not necessarily point to COVID-19 infection.[37] The suspected patients diagnosed for both IgM and IgG antibodies are more informative and can be useful for defining the stages of infection and prognosis. Considering the limitations, antibodies tests should not be completely relied upon for individual case testing. On a positive note, they are not expensive and can be performed quickly making them an excellent tool for evaluating the spread of the infection at the population level.[38] It is worth mentioning that subjects with IgG antibodies alone may not be enough for prevention of infection. It is also not clear whether infection will always lead to an antibody response and more importantly if it is enough for protecting against re-infection and for how long.[39] Another major issue is the detection of antibodies in immune-compromised patients like in one referred case where non-hodgkin lymphoma patient tested negative for IgG antibodies continuously for 12 weeks as evaluated by chemiluminiscent microparticle immunoassay technology.[40]

In antigen based tests, virus proteins are detected through antibodies in OP and NP swabs. Though, they are considered to be much more accurate than antibody based tests but still they also suffer from limitations like in few cases where virus load is less, they may miss the infection. In few cases, virus proteins might persist in the body and can give false positive antigen test even after complete recovery.[41]

2 | DISCUSSION

Most available diagnostic tests have limitations and are being continuously subjected to research and development for improving accuracy and precision (Figure 1).[30,42–45] The SARS-CoV-2 is rapidly evolving and undergoing mutations, and for ensuring efficacy, the local strains should be sequenced at regular intervals and checked for the mutations that can impact RT-PCR based diagnosis.[46] Recently, a point mutation has been identified in nucleoprotein gene of SARS-CoV-2 which resulted in false negative RT-PCR test.[46] Ziegler et al.[46] has suggested targeting of two independent essential region of SARS-CoV-2 for the accurate detection. A combination approach involving RT-PCR along with antibody and antigen test in complementation with chest X-rays and other symptoms looks most promising. Further, the call for conducting test again, should be taken on the case to case basis. As discussed before, there have been many instances where RT-PCR and antigen tests have missed the infection. In light of inaccuracies in diagnosis, proteomics based methodologies have been explored too like Whetton et al. described proteins involved in blood coagulation (like D-dimer), cell damage (such as lactate dehydrogenase), and the inflammatory markers (C-reactive protein) as the indicator of COVID-19 severity and mortality. Proteomics indeed has the excellent potential but high cost and the limited availability of the instrument can restrict its usage at large scale.[47] Further, there is no specific biomarker that can predict the severity of the disease. COVID-19 infection progresses in

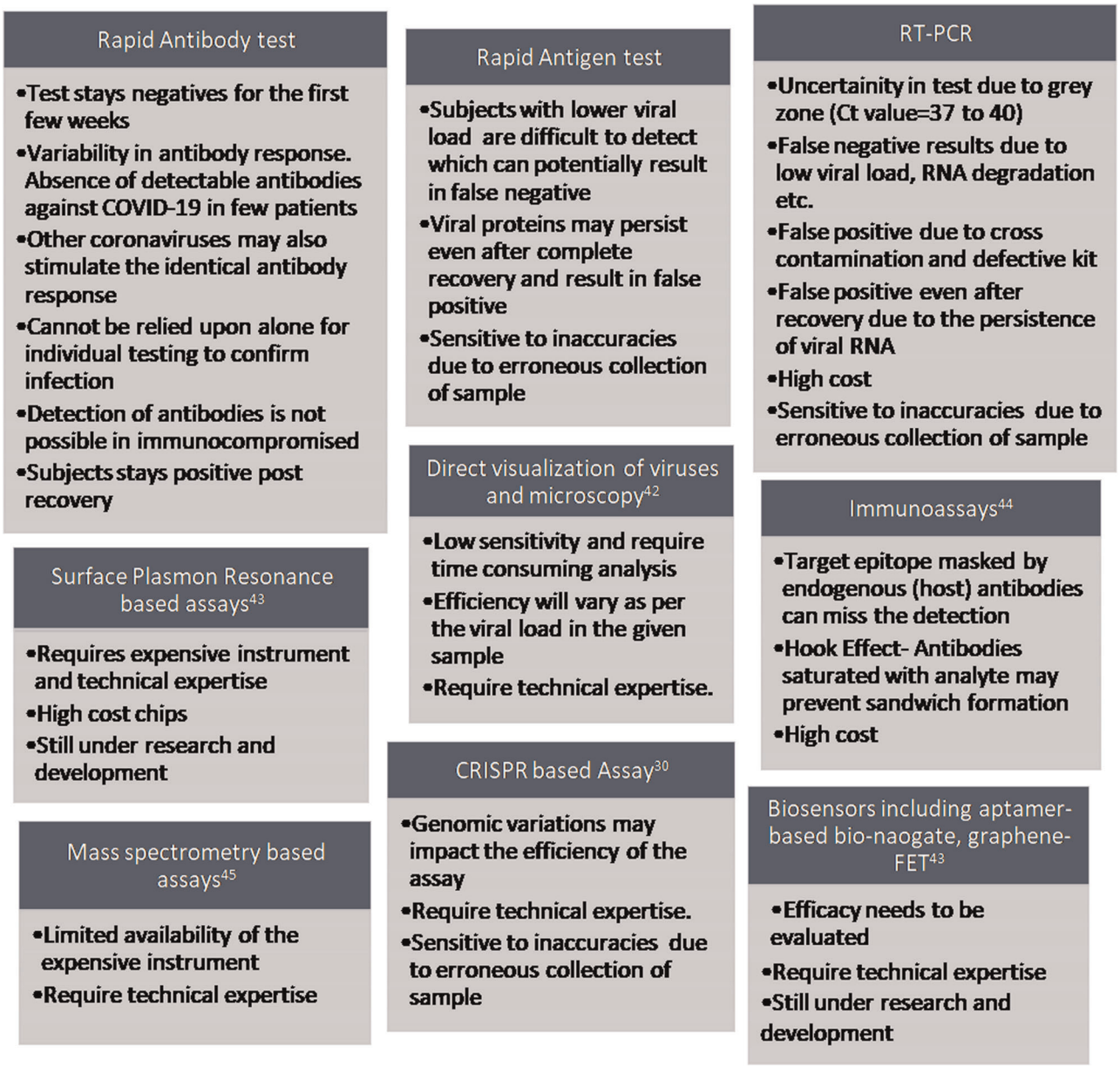

FIGURE 1 Sticky-note schematic presentation of limitations of diagnostic tools for COVID-19. COVID-19, coronavirus disease 2019. RT-PCR, reverse transcription polymerase chain reaction

different patients with diverse pace. The determinants of its progression are still not clear. The incubation period varies from 5.2 to 12.5 days and the diagnostic tests may not work in this duration.[48] Most patients remain asymptomatic and have lower viral load, and are difficult to diagnose. The emergence of COVID-19 threat has been rapid and diagnostic assays have been developed and tested on hospital samples that often contained more viral loads resulting in higher efficacy than in the real world.[29] On one hand, false negative tests can lead to the spread of COVID-19 in the community and on other hand, false positive tests are traumatic and lead to financial burden and psychological drain at the individual or family level, and affect policy making by portraying an over presentation of the epidemic.[29] New technologies like CRISPR based paper-strip,[49] Au/Ag nanoparticles based electrochemical biosensor, nucleic acid hybridization, aptamer-based bio-naogate, graphene-FET and surface plasmon resonance based detection hold immense potential as an improvised diagnostic tool in comparison to the traditional methods like RT-PCR and antibody assay but still they are in the development stage and need to be tested at a large scale as per necessary guidelines before utilizing them in real time.[43] Last but not the least, governments and health agencies should invest more on diagnosis, development of new methodologies and proper utilization of available tools more efficiently by combining them so that the final outcome of diagnosis becomes much more accurate than the individual one.

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