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Review Article

Laboratory Detection of SARS-CoV-2: Dilemma of Choosing the Best Test

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Abstract

The new coronavirus disease (COVID-19) pandemic is a global health problem that appeared in late 2019 through severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This perplexing virus has a high infection rate, and with no specific treatment, the mortality and morbidity rates are rapidly increasing. Moreover, the new virus variant, which is more contagious and has a higher mortality rate than previous variants, has been detected in the United Kingdom. There are few vaccines at clinical stages. However, the distancing, track, and trace will stay with us for at least the next few years. Hence, detecting symptomatic and asymptomatic patients through accurate detective tests such as molecular and serological assays and quarantine is the only preventive method that can be used for controlling the pandemic.

It is essential to use highly accurate tests to decrease the number of false negative and false positive results. This research aimed to highlight and critically assess the specificity and sensitivity of coronavirus tests available for detecting the SARS-CoV-2 virus. Currently, this is a multibillion-dollar industry, and many tests enter the clinical setting without having fully been validated.

Keywords: COVID-19; SARS-CoV-2; Laboratory Tests; Molecular Assays; Serological Assays; Vaccine; Pandemic

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Introduction

In late 2019, a COVID-19 outbreak with copious pneumonitis patients, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged in Wuhan, a city in the Hubei province of China. Although it was suggested that the patients infected with SARS-CoV-2 (1) In China, they might have utilized or visited infected

animals in the seafood market; further research revealed some patients with no history of seeing the seafood market. So, person-to-person virus transmission via coughing, sneezing, and aerosols that could permeate the lungs via the nose or mouth is unavoidable. (2, 3). Two strains of seven coronaviruses caused disease in humans. (4, 5) with zoonotic origination containing severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV) have been related to the occurrence of severe respiratory diseases in humans in 2003 and 2012 respectively (5). Now, the seventh member of the coronavirus family, SARS-COV-2, has led to an acute respiratory disease pandemic in humans. Due to its severe contagion, its rapid progression in morbidity and mortality rate, and the nonexistence of appropriate and one hundred percent effective vaccine and treatment, in addition to the appearance of the new, more contagious, and fatal variant of SARS-CoV-2 in the United Kingdom of Great Britain and Northern Ireland (6), the only solution for the management of preventive infection is social distancing and home quarantine. Therefore, detecting symptomatic and asymptomatic patients through diagnostic methods **(Table 1)** is essential. This research aimed to highlight and critically assess the specificity and sensitivity of coronavirus tests available for detecting the SARS-CoV-2 virus. This is valuable information for scientists and clinicians as well as the government for the management of the pandemic, as already crippling the people's health and economy.

SARS-CoV-2 entry mechanism

Coronavirus spike (S) protein has been shown to be a remarkable determinative factor in the entry of viruses into host cells (7). The entrance of SARS-CoV into cells is primarily performed via direct membrane fusion between the virus and plasma membrane (8). It has been reported (9) A significant proteolytic cleavage event happened at the SARS-CoV S protein at position (S2'), mediating the membrane fusion and viral infectivity. Alongside the membrane fusion, the clathrin-dependent and -independent endocytosis mediates the SARS-CoV entry as well (10, 11).

The SARS-CoV-2 S protein mediates the entry into cells **(Figure 1)**. To fulfill its function, the SARS-CoV-2 spike binds to its ACE2 receptor through its receptor-binding domain (RBD) and is proteolytically activated by human proteases (12).

Importance of the early detection of SARS-CoV-2

For managing the COVID-19 pandemic, despite observing the preventive points and investigating for finding and developing effective old and novel therapeutic methods, finding and developing appropriate diagnostic approaches is essential. As preventing the infection of a disease is better than treating it, there is a huge need for accurate diagnostic materials to find infected patients by SARS-CoV-2 in each stage of COVID-19, especially in the early stages of the disease to quarantine them and address their condition, especially with those patients with an underlying health condition. So, based on clinical and paraclinical manifestations, various diagnostic methodologies and appropriate samples with their accuracy are negotiated below.

Specimen obtaining and submission

Based on the disease development from mouth to anus, specimen sources of COVID-19 diagnosis are variable **(Figure 2)**. The determination of COVID-19 based on the stage of infection can be made by detecting nucleic acids of SARS-CoV-2 in specimens like nasopharyngeal and oropharyngeal swabs (13), lower respiratory tract secretions, stool, and blood **(Table 2)** (14). Between weeks 2 and 3 or 5-6 days after the inception of symptoms, the virus and its RNA culminate in COVID-19 patients' upper and lower respiratory tracts (15-17). For the most sensitive recognition of endemic SARS-CoV-2, upper respiratory specimens should be accumulated after the first few days of symptom inception or after the incubation period (Table 2). Upper respiratory specimens are accessible to accumulate; in addition to that, they enhance access to experiments for COVID-19 patients with mild symptoms and resource-limited settings. Therefore, in the early stage of COVID-19, throat samples are commonly tested positive. Although oropharyngeal (OP) swabs were applied more commonly than NP (nasal nasopharyngeal) swabs

in China during the COVID-19 outbreak, the SARS-CoV-2 RNA was recognized solely in 32% of OP swabs, which was significantly lower than that in NP swabs (63%) (18). Besides, NP swab is endured by the patients and is more noxious for health care workers. Therefore, an NP swab is the preferred specimen for detecting SARS-CoV-2 infection (16). The patients with pharyngitis as a prominent primary presenting symptom should be sampled via the OP. It should be taken into account that for properly obtaining an NP swab sample, the swab has to be entered profoundly into the nasal cavity and must be retained in place for 10 seconds while rotating three times, which results in a flinch, gag reflex, and evokes "tears." Besides, this sample-collecting method may hurt respiratory tracts, and cause bleeding, and due to the airborne transmission of SARS-CoV-2, it is unsafe for healthcare workers (19). Recently, the live virus was detected in the self-collected saliva and tears (along with viral conjunctivitis) of cases infected with SARS-CoV-2 (20-22); saliva and tear can be employed as a non-invasive specimen for the diagnosis of COVID-19 as an alternative method for assembling an upper respiratory tract sample (23, 24) with fewer transmission risks for healthcare workers and injury to the patients. Besides, it should be mentioned that the new Rutgers saliva test for coronavirus has been approved by the FDA (Food and Drug Administration) (25).

Note that in some COVID-19 patients, saliva, NP, and OP might miss early infection. So, alternative repeated testing could be applied over overtime to enhance the likelihood of the SARS-CoV-2 being present in the nasopharynx, or a lower respiratory tract specimen, including sputum and bronchoalveolar lavage (they are more invasive than upper respiratory tract sampling) should be obtained during the intubation scheme (15).

Interestingly, although ten days after the onset of symptoms, the throat samples tested negative, the anal swab was examined positive (26). So, the anal swabs gave more positive results compared to the oral swabs in the later stages of the infection (26). Hereupon, for up to five weeks **(Figure 3)**, after a mean of 11·2 days of respiratory tract samples became negative, viral shedding in the stool probably occurred. The RNA of SARS-CoV-2 is detected (27); due to the possibility of fecal-oral transmission, clinicians have to be wary when discharging any COVID-19 patients based on negative oral swab test results. The number of PCR-positive tests in various samples in patients with COVID-19 (18) was respectively, bronchoalveolar lavage (93%), sputum (72%), nasal swab (63%), bronchoscopic biopsy (46%), throat swab (32%), anal swab (29%), blood (1%), urine (0%) and serum (15% of patients hospitalized with pneumonitis) (17). Therefore, although bronchoalveolar lavage and sputum collecting may hurt the respiratory tract, they are the most valuable specimens. A single upper respiratory tract specimen, including a nasopharyngeal swab (NPS) or viral throat swab accumulated in a universal transport medium (UTM), is accepted for patients not admitted to the hospital. However, NPS is the preferred specimen, and in patients admitted to the hospital, collecting upper and lower respiratory tract samples is advised when feasible. Thus, for the most sensitive detection of SARS-CoV, MERS-CoV, and SARS-CoV-2 in early stages, the gathering and testing of both upper and lower respiratory specimens and, in late stages, lower respiratory and stool specimens are recommended. (17). After collecting samples, they must be placed in a biohazard bag sealed at 2-8°C and dispatched to the destination lab on ice packs as soon as possible. If the transport of samples to the destination lab lasts more than 72 hours, samples must be frozen at -20°C or ideally -70°C or -80°C and shipped on dry ice. (28, 29).

Diagnostic methods for COVID-19

Physical examination, chest CT, chest X-ray, and laboratory diagnostic methods are the standard diagnostic methods of SARS-CoV-2 **(Figure 4)**. In general, there are three laboratory diagnostic techniques, including molecular tests (Nucleic Acid Amplification Tests (NAATs and viral sequencing), serological testing, and viral culture. Significantly, most of these assays have been approved solely for research, which suggests that they are not yet approved as a public health diagnostic device or for at-home diagnosis.

Molecular assays

RT-PCR

Although RT-PCR is the gold standard due to

its accuracy, the slow speed and difficulty of sample collection are weaknesses. Currently, NAATs are most commonly used in the diagnosis of COVID-19. The most common method used in this group is real-time reverse transcriptase PCR or rRT-PCR.

Formerly, conventional PCRs only provided us with an end-point analysis of the pathogen at the end-point of the run. However, new PCR devices, especially reverse transcriptase PCR devices, can be qualitative or quantitative, which allows us to trace live and amplify viruses at different times. In the qualitative state, it shows whether the sample is infected with the pathogen or not, but for determining the viral load, quantitative PCR (qPCR) or real-time PCR is preferred.

Note that SARS-CoV-2 is an RNA virus, so for producing usable cDNA for PCR, reverse transcriptase is necessitated (30). The target genes in RT-PCR are spike (S) protein, envelope (E), transmembrane (M), helicase (Hel), nucleocapsid (N), RNA-dependent RNA polymerase (RdRp), hemagglutinin-esterase (HE), and open reading frames ORF1a and ORF1ab. (20, 28, 31). However, the CDC recommended two nucleocapsid protein targets. (32), including N1 and N2, WHO recommended first-line screening with the E gene assay followed by a confirmatory test applying the RdRp gene. However, it must be mentioned that the sensitivity of the RNA-dependent RNA polymerase gene of the SARS-CoV-2 viral genome as a corroborative assay is insufficient, and applying the N gene is better (69). So, other detective methods like serological and radiological detective methods are necessary for COVID-19 infection confirmation. It must be mentioned that more than 200 nucleic acid detection kits have been developed (33), like the supreme pure viral RNA kit (Roche), HiScript® II 1, one Step qRT-PCR SYBR® Green Kit (Vazyme Biotech Co., Ltd) (66). Moreover, FDA approved PerkinElmer's real-time RT-PCR, which is compatible with a variety of sample types (73).

Currently, polymerase chain reaction (PCR) and antibody testing are the dominant methods of global health systems to test citizens for COVID-19. Both techniques have their precautions, and as the crisis unfolds, scientists are looking for alternative methods to screen for the disease.

Detecting the RdRp gene or any other single gene or several genes through RT-PCR or detecting viruses through sequencing is adequate for laboratory confirmation (28, 29). If one or multiple targets is/are indeterminate through RT-PCR and the virus is not detectable via sequencing, laboratory testing will be considered inconclusive. Moreover, when one or more specimens are tested negative, clinicians and scientists must not rule out the feasibility of COVID-19 virus infection. However, they must repeat the tests or adopt other diagnostic methods (like serological tests (SARS-CoV-2 specific antibodies and antigens) and radiological imaging).

RT-qPCR is required for precise and reproducible quantification (34). It is essential for diagnosing COVID-19 in almost all stages of the disease, especially in the early stages. For discharging COVID-19 patients and finding an appropriate donor in plasma therapy, two sequential negative COVID-19 nucleic acid identification at least 24 hours apart (35, 36) Is needed. It is considered as a practical approach for confirming the diagnosis in clinical cases of COVID-19 (78). Besides, over seven types of SARS-CoV-2 nucleic acid test kits have been extended and approved rapidly (78, 79). Through point-of-care tests that do not require to be done in the lab, the turnaround time (TAT) is significantly reduced. In this regard, Cepheid has developed a point-of-care test called Xpert® Xpress SARS-CoV-2 that gives a qualitative rRT-PCR analysis within 45 minutes (target genes: E, N, RdRp, and ORF1a) (28). Moreover, Abbott has also developed a point-of-care qualitative PCR test called ID NOW COVID-19 that reports the positive rRT-PCR in 5 minutes and the negative response in 13 minutes (target gene: RdRp) (37).

It's worth noting that PCR tests can be very labor-intensive, with several stages at which errors may happen between sampling and analysis. It has about 28 steps (30), in which the turnaround time (TAT) for testing eight specimens by RT-PCR is about 7-8 hours to even 24 hours. So, it is a time-consuming test and is not appropriate for emergency detection of a large population like the size of the world population (28).

Moreover, the virus may not be diagnosed (false negative) due to the restriction of sampling materials, specifically in the early stage of the disease, and inaccurate specimen accumulation based on

the COVID-19 development (small viral load). There are several other reasons the patient arrived later or sooner than a specific time (low viral load), the specimen was not handled and shipped appropriately (29), and technical problems (38-41). Although RT-PCR is a reverse transcription method, the saliva swabs applied to collect the clinical samples should be immediately added to lysis buffer to disinfect the specimen as well as to prevent the destruction of the coronavirus RNA. (42, 43), the clinical samples must be warmed to 56oC for 30 minutes, which may destroy the coronavirus RNA (43). It must be noted that to avoid probable cross-reaction (false positive) with other endemic coronaviruses and potential genetic drift of SARS-CoV-2 as well, at least two molecular targets must be included in the test, which is time-consuming and it is not cost-effective. It must be mentioned that this method is associated with unnecessary hazards to healthcare workers due to close contact with patients. (24). The accumulation of sputum and particularly bronco alveolar lavage (BAL) via bronchoscopy enhances biosafety peril to healthcare workers through the production of aerosol droplets. By means of RT-PCR, it is not possible to retrospectively figure out who is sick (for example, asymptomatic patients or patients with mild symptoms) or who is potentially safe.

RT-PCR must be performed under a particular condition in the central laboratory (as RNA is sensitive and vulnerable), like in a negative pressure room, by experts (34, 38). So, it cannot be performed everywhere by inexpert persons. In addition, there are several technical problems. (38-41) Using RT-PCR, which one of them is as the PCR perfectly amplifies the target, errors are increased as well. By considering all aspects of RT-PCR, it is a gold standard test for detecting COVID-19 cases.

Other Developing Molecular Assays

Some of them can be practical detective approaches to SARS-CoV-2. They are composed of next-generation sequencing and metagenomics, Sanger sequencing, Whole-genome sequencing, CRISPR Cas13, multiplex isothermal amplification, and closed-tube Penn-RAMP.

Random-amplification deep-sequencing methods, which are composed of Next-generation sequencing and metagenomics, are required to identify future mutations of SARS-CoV-2 but are inappropriate for diagnosing COVID-19 infections. So, at this time, they are not practical for detecting SARS-CoV-2. Sanger sequencing is used for the RdRp gene to diagnose the disease, especially when the PCR results for a target gene are not convincing. Still, the clinical/epidemiological suspicion of COVID-19 is high. (28).

Whole-genome sequencing is used less frequently in diagnosis. Still, it is applied to find genomic correlations between patients (molecular epidemiology) and the probability of mutations with MinION Nanopore materials.

It must be mentioned that CRISPR Cas13 can be effective for diagnosing SARS-CoV-2 infection. In one study, applying the CRISPR-based SHERLOCK (Specific High Sensitivity Enzymatic Reporter UnLOCKing) method for detecting SARS-CoV-2 is recommended. However, further investigations are essential (44). Multiplex isothermal amplification, accompanied by microarray identification, is another molecular method that is being extended and appraised worldwide. (45).

As Closed-Tube Penn-RAMP can significantly decrease false-negative results of RT-PCR, has low cost and high sensitivity, and can be applied as an at-home diagnostic method, it has been adopted by researchers (46). This test is composed of two isothermal amplification processes, including Recombinase Polymerase Amplification (RPA) and loop-mediated isothermal amplification (LAMP). As this test requires the least sample processing, it can be simply applied at home.

Serological detection

In contrast to RT-PCR, serological assays looking for signs of the virus in blood can result in less than an hour and need only a specimen finger prick. These assays search for evidence of an immune response to the infection – not the virus itself – by testing for antibodies that combat the coronavirus in a patient's blood (47).

Most antibody tests look for evidence of the "first responder" antibodies IgM that appear about a week after infection and IgG antibodies that are longer-lasting antibodies created two to four weeks after infection. Several studies have revealed that people who survived the SARS-CoV

outbreak had antibodies in their blood for years after recovery. It is not certain that SARS-CoV-2 can produce a similar immune response, as it has been indicated that some people have been infected with the virus twice, suggesting that these patients didn't develop any immunity at all. (47). Although this evidence suggests that serological testing is not accurate, it is used in the detection of COVID-19.

Serological tests contain enzyme-linked immunosorbent assay (ELISA) or Western blots that distinguish specific COVID-19 proteins (48), neutralization assay (including IgM, IgG, IgA antibodies against viral antigens such as the nucleoprotein and the receptor-binding domain of the S protein), and rapid diagnostic test (RDT).

Rapid diagnostic tests (RDTs)

They are composed of rapid antigen tests and immunoassay techniques. As these tests are resembling pregnancy assays, blood samples from a finger prick, saliva specimens, or nasal swab fluids are suitable. So, these can be the preferred diagnostic method for COVID-19. For COVID-19, these tests are usually used to test the patient's antibodies (IgG and IgM) or viral antigens. In China, six serology detective devices have just gained immediate approval from the National Medical Products Administration (NMPA) by March 12, 2020. Also, there is a clinical trial for serological detecting of SARS-CoV-2 named Clinical Performance of the VivaDiag ™ COVID-19 IgM / IgG Rapid Test in Early Detecting the Infection of COVID-19 (NCT04316728). It must be mentioned that in some patients, it could be advantageous to measure IgG and IgM titres before infection.

Rapid antigen tests

FIND organization (https://www.finddx.org/) has listed ten CE-marked (it is a certification mark that proved conformity with health) quick SARS-CoV-2 antigen detection tests. Besides, multiple rapid antigen tests are being extended (https://www.medrxiv.org/content/10.1101/2020.03.07.20032524v1. Assessed March 13, 2020). They will make the possibility of determining a load of infection in asymptomatic patients. So they can be appropriate detective methods for COVID-19 disease. Although rapid antigen tests would probably make the benefits of quick time to results and low-cost detection of SARS-CoV-2, due to the low burden of virus or sampling variability, they have low sensitivity and may miss COVID-19 cases. Although the interpolation of colloidal gold-labeled IgG as the detective reagent is a method that might enhance the sensitivity of rapid antigen tests for respiratory viruses (49), they are not preferred for COVID-19 diagnosis.

Immunoassay techniques

They are composed of fluorescence immune chromatographic assay, Magnetic Chemiluminescence Enzyme Immunoassay, Colloidal Gold-Immunochromatographic Assay (GICA), and Lateral Flow Assays (LFAs).

A peptide-based Magnetic Chemiluminescence Enzyme Immunoassay, which is an accelerated diagnostic test, has been used for COVID-19 diagnosis. Results indicated that the simultaneous application of this assay and real-time RT-PCR might increase the sensitivity of COVID-19 detection (50). One study offered that a fluorescence immune chromatographic assay is a precise, rapid, early, and secure method for detecting nucleocapsid protein of SARS-CoV-2 in NP swab for diagnosis of COVID-19 infection (17).

Colloidal Gold-Immunochromatographic Assay (GICA)

Colloidal Gold-Immunochromatographic Assay (GICA) relies on specific antigen-antibody immunoreactions, and its turnaround time is 30 minutes without any expertise requirement. Therefore, it is beneficial for the early detection of COVID-19 infection. It must be mentioned that rapid GICA kits for detecting anti-SARS-CoV2 IgM and IgG antibodies have been used for 91 serum specimens of 91 suspicious COVID-19 patients (51). Although, due to remarkable numbers of false-negative results, it has not been used commonly; it has a sensitivity and specificity of 86.89% (106/122) and 99.39% (656/660), respectively. Moreover, 21of 32 clinically confirmed COVID-19 patients with negative RT-PCR results were GICA positive. Besides, simultaneously application of real-time RT-PCR detection and GICA sandwich detected total antibodies might increase the sensitivity of detection (52). So, it can be effective for diagnosing SARS-CoV-2.

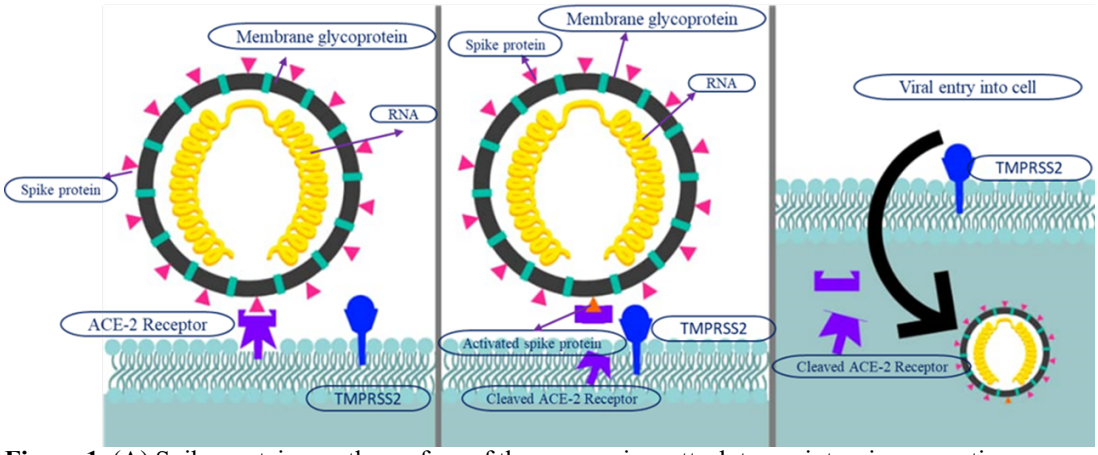

Figure 1. (A) Spike proteins on the surface of the coronavirus attach to angiotensin-converting enzyme 2 (ACE-2) receptors on the surface of the target cell; (B) The type II transmembrane serine protease (TMPRSS2) attaches to and cuts the ACE-2 receptor.

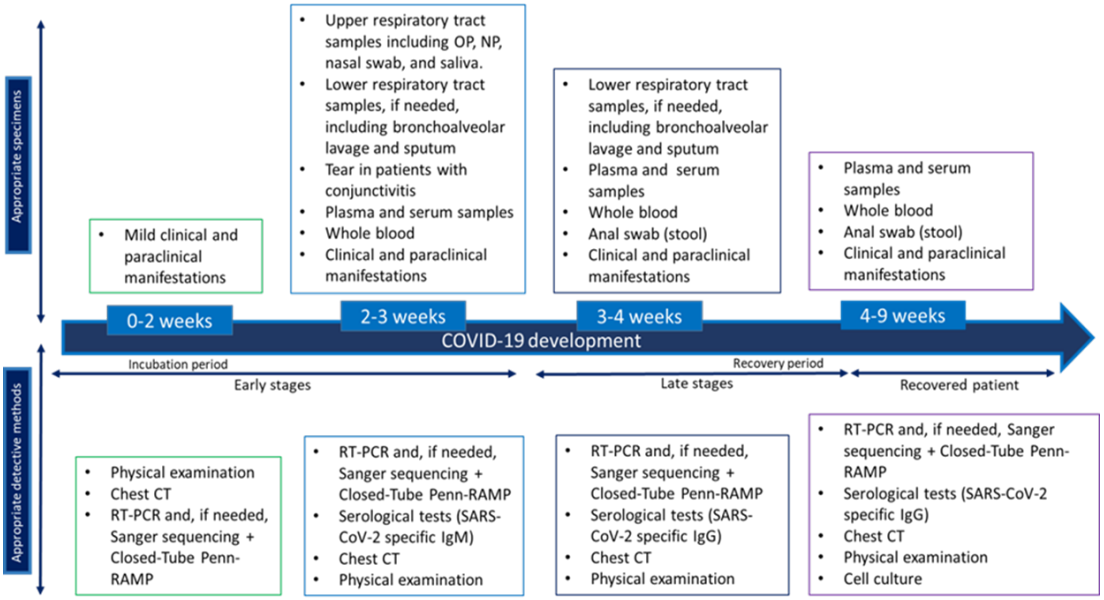

Figure 2. Appropriate specimens and detective approaches of COVID-19 based on its development.

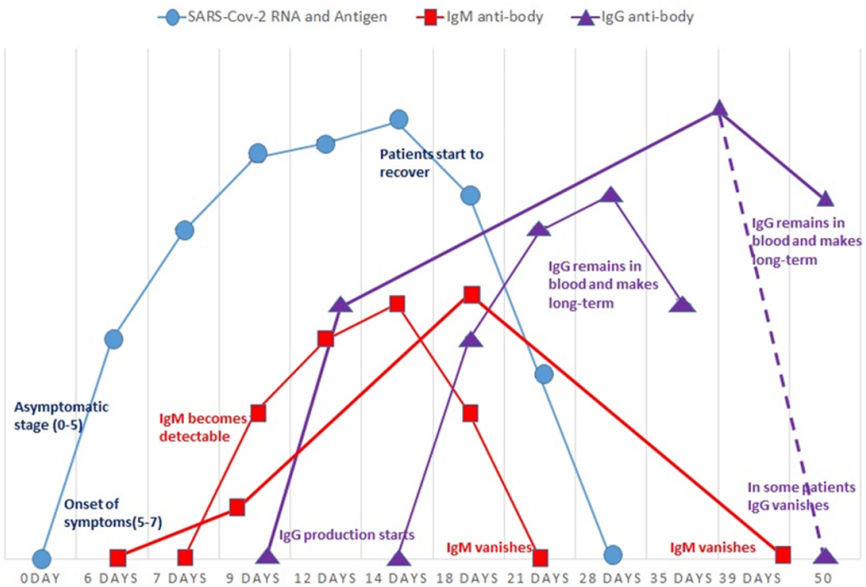

Figure 3. Serological changes during COVID-19 development. Just for the illustrative objective.

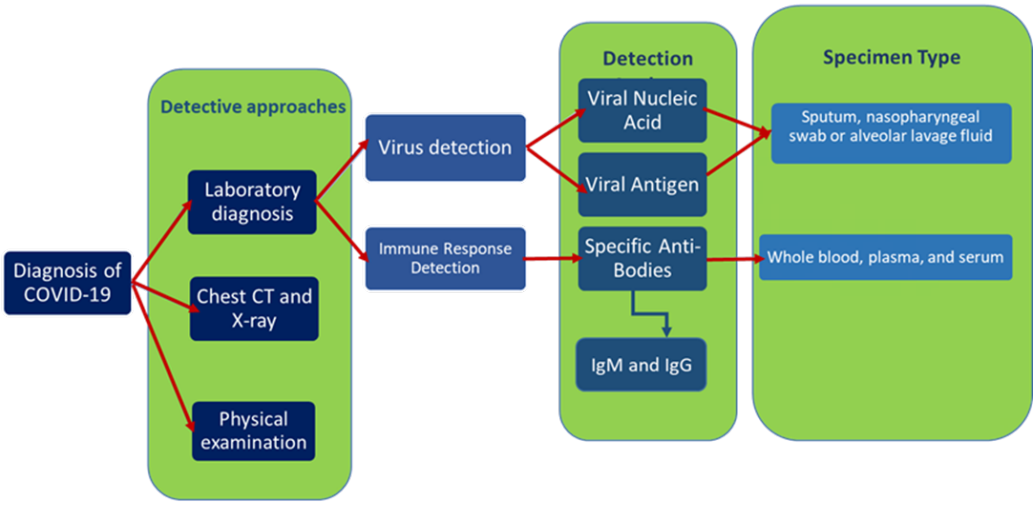

Figure 4. Diagnostic approaches of COVID-19.

Table 1. SARS-CoV-2 diagnostic tests

Preclinical	Clinical	Radiological	Physical examination
Next-generation sequencing and metagenomics	RT-PCR	Chest CT	Medical history
Neutralization assays	point-of-care tests	chest x-ray	Fever
Sanger sequencing	Rapid antibody tests (RDTs)		Sore through Dry cough
Whole-genome sequencing	Enzyme-linked immunosorbent assay (ELISA)		Tiredness Aches and pains
CRISPR Cas13	lateral flow assay		Anosmia and ageusia
Multiplex isothermal amplification cell culture	colloidal assay Immunochromatography Colloidal Gold-Imtographic Assay (GICA) Rapid in-clinic antigen testing		Vomiting, nausea, and diarrhea Conjunctivitis Headache A rash on the skin or discoloration of fingers or toes Difficulty breathing or shortness of breath Chest pain or pressure Loss of speech or movement

Lateral Flow Assays (LFAs)

Lateral Flow Assays (LFAs) are rapid antigen tests. They are cost-effective, secure, prompt, and movable detection tools that are commonly employed for qualitative, semi-quantitative and somewhat quantitative monitoring in non-laboratory circumstances (91) which Sona Nanotech (Halifax, Canada) is developing a quick-response lateral-flow assay to screen COVID-19 patients targeting to prepare results in 5–15 min (92). They are current point-of-care (53) diagnostic strategies that detect both IgM and IgG antibodies and have an essential role in COVID-19 (93). So, they can be used for diagnosing SARS-CoV-2. It must be mentioned that the Biopanda COVID-19 lateral flow immune-chromatographic rapid test qualitatively detects IgM and IgG antibodies to SARS-CoV-2 in whole human blood, serum, and plasma specimens (54).

Also, its turnaround time is 10 minutes; results could be read visibly; there is no requirement for an analyzer, and it is a cost-effective method for diagnosis the COVID-19.

However, this COVID-19 accelerated test must not be applied until the onset of symptoms for at least three days.

Table 2. COVID-19 diagnostic assays.

Laboratory assays							
Unavailable assays (Preclinical) **Next-generation sequencing and metagenomics, Sanger sequencing, Whole-genome sequencing, CRISPR Cas13, Multiplex isothermal amplification, Neutralization assays, and cell culture.**							
Available assays (Clinical)							
Lab test	**Methodology**	**Specimen**	**Minimum Valium**	**Collection material and kit**	**The turnaround time**	**Cost**	**accuracy**
RT-PCR **point-of-care tests of Cepheid** **The point-of-care test of Abbott Closed-Tube Penn-RAMP**	It is used to diagnose people who are currently sick with COVID-19. The genetic material of the coronavirus in mucosal samples can be looked for by amplifying the viral genetic material if it is present (65).	Upper respiratory tract: NPS NPS, OPS or wash in ambulatory patients, and viral throat swab (28, 29). Lower respiratory tract (when possible): endotracheal aspirate, sputum, BAL (bronchoalveolar lavage), pleural fluid, lung tissue (see Submission and Collection Notes below) (28, 29). Viral nasal swab (66). Viral oral swab (20). Viral anal swab (28, 29). Viral tear swab (22).	Nasopharyngeal swab in 1 ml universal transport Swab in 1 ml universal transport media (UTM) Swab in 1 ml UTM Swab in 1 ml UTM Swab in 1 ml UTM Swab in 1 ml UTM	Virus Respiratory Kit Virus Culture Kit Tuberculosis Kit order container container container container	13 minutes (67) to several days (65) The point-of-care test of Cephoid results in 45 minutes. The point-of-care test of Abbott results in positive rRT-PCR in 5 minutes and a negative response in 13 minutes.	From $3,623 to$3,911 (68).	RT-PCR is the most reliable and gold standard test because of its accuracy. 100% sensitivity on positive samples (69) at least 96% specificity on negative samples (69) The real-world clinical sensitivity 66% -80% (69, 70).
Rapid antibody tests (RDTs) **Enzyme-linked immunosorbent assay (ELISA)**	They are blood tests. They look for antibodies to the coronavirus after four days to more than a week after infection and identify people previously infected with SARS-CoV-2. (65).	Blood samples from a finger prick Saliva specimens Nasal swab Serum, and plasma (28, 29).	Swab in 1 ml UTM 3-5 ml whole blood finger-prick blood	Container Serum separator Collection tubes	A few minutes (based on a drop of blood taken from the finger) IgM tests 10-15 minutes. IgG tests up to 7 days (71).	It must be mentioned that various tests cost €300 - €1000 (72).	90%. Sensitivity and 95%-99% specificity -positive rate less than 5% (47). Abbott at-home rapid tests are 98.5% sensitivity and 99.5% specificity (73). Enzyme-linked immunoassay (ELISA) is the most accurate test but is not as widely available.
Immunoassay techniques lateral flow assay colloidal assay immunochromatography	Immunoassay methods have been extended for quick detection of SARS-CoV-2 antigens or antibodies.	Urine Saliva Sweat Serum Blood and other fluids	Swab in 1 ml UTM 3-5 ml whole blood	Container Serum separator tubes Collection tube	5 to 15 minutes (70).	less than 50$ (70). There are several other assays, which cost from 100-500 (74).	. Accurate to be used as an in-home test (70)
Colloidal Gold-Imtographic Assay (GICA)	It relies on specific antigen-antibody immunoreactions.	Urine Saliva Sweat Serum Blood and other fluids	Swab in 1 ml UTM 3-5 ml whole blood	Container Serum separator tubes Collection tube	30 minutes	Low cost	86.89%sensitivity 99.39% specificity
Rapid in-clinic antigen testing	It identifies viruses in nose and throat secretions by identifying the virus proteins. It is used as a rapid test to detect individuals who are currently infected with SARS-CoV-2 (65). Moreover, it may be applied to screen people to identify those who need a more definitive test.	Urine Stool Saliva, Sweat, Serum Upper and lower respiratory samples Blood and other bodily fluids	Swab in 1 ml UTM 3-5 ml whole blood	Container Serum separator tubes Collection tube	2.5 hours (70) or even a few minutes (65).	It costs less than PCR tests (75).	85%,sensitivity 100%.specificity (65).
Radiological assays **Chest CT, chest x-ray**							
Physical examination							

Keys: NPS, Nasopharyngeal swab; OPS, oropharyngeal swab

Enzyme-linked immunosorbent assay (ELISA)

It is a lab-based assay that could be qualitative or quantitative. It generally applies whole blood, plasma, or serum specimens from patients. It depends on a plate that is covered with a viral protein, like spike protein. For COVID-19 patients, these tests are most commonly used for detecting patients' antibodies (IgG and IgM) against SARS-CoV-2. In-house anti-SARS-CoV IgG and IgM ELISA kits were extended applying SARS-CoV Rp3 NP as an antigen, that portioned over 90% amino acid identity to all SARS-CoVs (55). For the IgG test, MaxiSorp Nunc-Immuno 96 well ELISA plates were covered (100 ng/well) overnight with recombinant NP, and for the IgM test, MaxiSorp Nunc-Immuno 96 well ELISA plates were covered (500 ng/well) overnight with anti-human IgM (μ chain) (26). It must be mentioned that one recent study indicated that the sensitivity of the combined ELISA IgM and ELISA IgG identification and combined GICA IgM and GICA IgG detection was respectively 55/63

Table 3. Comparison of molecular and serological assays sensitivity during COVID-19 development.

Clinical importance of various stages of COVID-19	Assays consequences		
	IgM	IgG	RT-PCR
The patient in the active phase of the SARS-CoV-2 infection	+	+	+
The patient might be in the first stage of COVID-19	+	-	+
The patient might be in the early stages of COVID-19 (RT-PCR may be false-negative)	+	-	-
The patient might be in the late or recurrence stage of the COVID-19	-	+	+
The patient might be in the incubation period of the COVID-19	-	-	+
The patient might be in the recovery period of COVID-19 with false-negative RT-PCR	+	+	-
Recovered patient of the SARS-CoV-2 infection	-	+	-

(87.3%) and 75/91 (82.4%), and results suggested that as both of them are simple, fast, and safe. So, they might be applied for clinical reference and the enormous clinical diagnosis (51).

Neutralization assays

They are lab-based methods that rely on cell culture and allow SARS-CoV-2 growth. They detect patient antibodies to prevent viral infection of cells in a lab setting. They use the whole patient's blood, serum, or plasma specimens. They can indicate to clinicians and researchers whether patient antibodies are active and effective against the virus or not and can show whether the patient is protected against future infection or not. In one study of 175 recovered patients discharged from hospital in Shanghai, not only in one-third of patients, the levels of COVID-19 antibodies were unexpectedly low, but also in someone, antibodies could not be detected at all **(Figure 4)** (56). So, as there is a potential higher risk of reinfection, neutralization assays might have a useful role.

One study proved that both IgM and IgG antibodies were recognized five days (Figure 4) after inception in all 39 cases of COVID-19 in the study (57). In zero-day of COVID-19, SARS-CoV-2 specific IgM and IgG were detected in some patients, but after five days (Figure 4) in 100% and 81% of patients, IgG and IgM were detected respectively (26). In these conditions, COVID-19 cases may not be tested positive for viral RNA, especially in the early phase of the disease. So serological detection might be effective in the initial period of COVID-19. It must be mentioned that although the SARS-CoV-specific IgM persisted at a high level for about two weeks, the SARS-CoV-2-specific IgM remains at a detectable level for about nine days then rapidly reduces at day 39 (Figure 4) after the inception of COVID-19 symptoms (58). Also, the creation time and the development time of COVID-19 specific IgG after its inception are 9 to 12 and 9 to 39 days (Figure 4) (58). Thus, although changes in SARS-CoV-2-specific IgM and IgG vary from study to study (Figure 4), they are appropriate for early detection of the acute phase of COVID-19 and for the epidemiological study of that, respectively. Besides, this point must be taken into account that like severe cases of SARS-CoV, severe cases of SARS-CoV-2 infection have shown earlier and higher levels of IgM and IgG than mild cases (58).

When SARS-CoV-2 was not detected through RT-PCR or when rapid antigen testing and molecular assays are neither available nor stable, serology could be applied as an additional diagnostic device. Moreover, although along with COVID-19 development, there is a reduction in the number of positive tested molecular assays of the throat and anal swabs, there is an enhancement of positive tested serological assays (26). So, simultaneously application of serological assays with other diagnostic tests for confirming the COVID-19 diagnosis is recommended.

Serological assays play an essential role in the investigation of an ongoing outbreak. It is necessary for figuring out the epidemiology of emerg-

ing SARS-CoV-2, containing the burden and function of asymptomatic infections.

Retrospective assessment of extent or the attack rate of an outbreak is possible with serological detection. When rapid antigen testing and NAAT assays are negative, but there is a robust epidemiological connection to COVID-19 infection, paired serum samples (in the acute and recovering phase) could confirm diagnosis once credited serology tests are available (29). Serum samples could be stocked for these purposes. It has been advised to apply serological detective approaches to promote the detection of SARS-CoV-2 infections when an NP swab sample was accumulated incorrectly, and the molecular tests were conducted inappropriately (57). There is no expertise needed to diagnose COVID-19 through serological assays, and the protocol for using them is available online. So, they are accessible and cost-effective tests. Serological tests are sensitive and precise for the screening of COVID-19 seroconverts, implementing human plasma/serum as early as three days after symptom inception and can be utilized for a broad population. Significantly, serological detection is harmless for healthcare workers and does not need handling of contagious virus and could be adjusted to detect different antibody types and are acceptable to scaling. As serological tests are sensitive, antibody titers will support screening of health care workers to recognize those who are formerly immune and could be circumfused to care for COVID-19 cases reducing the threat of viral propagation to co-workers and other patients (59). Serological tests are imperative to identify highly reactive human donors for the generation of convalescent serum as a therapy. For a complete perception of the benefits and sensitivity of supplementary serological methods and molecular assays in the diagnosis of COVID-19 in different stages, and discharging suspected and confirmed COVID-19 patients, the comparison of the serological and molecular detective methods sensitivity in COVID-19 diagnosis in the various stages of the disease is located in **Table 3**. Although in the analytic phase, real-time RT-PCR analysis rests the choice among other molecular assays for the etiologic diagnosis of SARS-CoV-2 infection, antibody-based approaches are being applied as complementary tools in the post-analytical stage. So, testing results must be accurately interpreted, using both molecular and serological testing outcomes.

It must be mentioned that there are several disadvantages with serological assays like cross-reactivity to other coronaviruses that can be challenging, and they cannot apply early in human illness because antibodies may not yet be produced.

Cell culture

Viral culture is currently not recommended as a standard diagnostic method for SARS-CoV-2 due to the lack of permissive cell lines (MERS-CoV and SARS-CoV-2 will grow in primary monkey cells and cell lines like Vero and LLCMK2), time-consuming, and expertise necessities, and the absence of market antisera for culture passage. Besides, the cultivation method has more been used for research aspects like using experimental treatments to confirm the presence of a live virus at different levels, the degree of infection between individuals, development of vaccines and therapeutic agents, and the control of infection of cells cultured in the laboratory (17, 29). So, it is not applied as a standard diagnostic method for the clinical diagnosis of COVID-19.

Physical examination

According to the clinical manifestation of COVID-19, clinicians can probably distinguish COVID-19 among various diseases like Adenovirus, Influenza, Human meta PneumoVirus (HmPV), Parainfluenza, Respiratory Syncytial Virus (RSV), and Rhinovirus (common cold) (60). However, due to variable clinical manifestations and consequences in different patients in various stages of the disease, physical examinations must be accompanied by paraclinical diagnostic methods for confirming COVID-19 infection.

In terms of renal function collapse, one patient revealed severe renal function impairment (UREA: 26.5 mmol/L, CREA: 1054.4 mmol/L). It must be noticed that the blood glucose (GLU) level of 23.17% of patients passed the standard range, and the level of D-dimer was enhanced in 3.75% of cases.

Chest CT

Chest CT scan is a perfect diagnostic method for identifying viral pneumonia as the sensitivity of chest CT images was 97% with reference

RT-PCR (61), and the sensitivity of chest CT is far more superior to the X-ray. Moreover, in asymptomatic patients of COVID-19, lung CT scans have shown pneumonia (62). So, like rapid serological methods for rapid early diagnosis of COVID-19 in a large population, chest CT is appropriate (63).

SARS-CoV-2 is severely contagious, clinical consequences are variable, and currently, there is no special treatment and effective vaccine. The only solution forcontrolling the mortality and morbidity rate of the pandemic is detecting COVID-19 patients through molecular and serological assays along with using radiological assays based on the stage of the disease and appropriate specimens.

Although NP, instead of OP swab is recommended for early diagnosis or screening as it makes better diagnostic accuracy and better tolerated by the patients. However, this may not be safe for healthcare workers. Therefore, self-collected saliva or nasal washes can be used as alternative methods of specimens' collection in the early stages of the disease. In COVID-19 development, the upper respiratory tract samples may test negative, which suggested that the sample should be taken from deep sputum or. The repeated testing or the bronchoscopy application for patients highly suspected of COVID-19 based on the epidemiologic history, symptom, laboratory examination, and CT findings is recommended. In the late phases of the disease, the role of rectal swabs is crucial.

Although real-time RT-PCR is a time-consuming method and has a high rate of false-negative results, which is not appropriate for early detection of COVID-19 in a large population, it considered a "gold standard" diagnostic technique. However, some companies like Abbott and Cephoid have produced a rapid point of care tests. Moreover, the low sensitivity of RT-PCR might be solved through its combination with Colloidal Gold-Immunochromatographic Assay (GICA) or peptide-based Magnetic Chemiluminescence Enzyme Immunoassay. Besides, because of possible cross-reactions, which result in false tests, serological assays should be confirmed by RT-PCR. Serological assays with high sensitivity and specificity rates have been extended as quick diagnostic tests based on identifying SARS-CoV-2-specific antibodies. Also, contrary to molecular tests, serological assays could be used for epidemiological surveys, and there are fewer risks for healthcare workers than molecular assays. Rapid antigen tests and Lateral fellow assays are some of the most rapid, cost-effective, sensitive, and specific serological assays. ELISA is also considering the most sensitive serological assay; it is not rapid, cost-effective, and easy-to-use for a large population. However, serological tests like rapid antigen tests can easily be applied as an at-home diagnostic method of COVID-19 since there is no need for expertise equipment, and they are comfortable and cost-effective assays. Moreover, it must be noted that the portable smartphone-based SARS-CoV-2 testing kit produced by UK scientists can be used as an at-home test.

Neither PCR nor serological tests are complete. However, they are far better than nothing and provide tremendous valuable information to medical professionals, public health professionals, and people who are being tested (47).

Conclusion

Currently, serological tests are not recommended for COVID-19 detection. However, they will play a role in research and surveillance. Rapid Diagnostic assays are not currently recommended for clinical diagnosis or triage and investigation of clusters pending more evidence on test performance and operational utility they require to be evaluated. COVID-19 antibody detection serology tests (Immunoassays, ELISA, IFA, and RDTs) are not recommended by WHO for the identification of SARS-CoV-2 infections. For COVID-19 diagnosis, WHO has recommended NAAT testing on respiratory tract specimens (64).

Applications of COVID-19 antibody detection assays (including RDTs) are currently restricted to seroprevalence investigations and retrospective diagnosis in NAAT-negative individuals presenting late during COVID-19 infection. Moreover, the WHO recommends not to interpret the presence of COVID-19 antibodies as "protected against reinfection" (64).

All in all, SARS-CoV-2 specially its new variant in United Kingdom is very contagious and fatal. Although several vaccines are at clinical stage, for controlling the pandemic there is a huge need for the usage of the most accurate test with the lowests false ngative and false positive results.

Finally, despite the requirement of using multiple methods for increasing the sensitivity and specificity rates of detecting SARS-CoV-2 in each stage of COVID-19, RT-PCR along with supplementary serological methods for increasing the sensitivity of the detection is preferred approaches with appropriate specimens.

Conflict of interest

The authors have no conflicts of interest.

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