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COVID-19 A PANORAMIC VIEW (PART 3)

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ABSTRACT: SARS-CoV-2 infection or COVID-19 exhibits a wide range of clinical symptoms; from mild flu-like symptoms to severe respiratory distress syndrome. Identification of COVID-19 patients at early stages will allow prompt intervention for patients with life-threatening complications. Broad categorization of the testing modalities results in 2 major types of tests- molecular assays for detection of SARS-CoV-2 viral RNA using polymerase chain reaction (PCR)-based techniques or nucleic acid hybridization-related strategies. Viral RNA testing identifies infected individuals during the acute phase of infection. Serological and immunological assays are included in the second category and they largely depend on detection of antibodies. RT-PCR-based viral RNA detection can neither be used to monitor the progress of the disease stages nor can be applied for identification of past infection and immunity. Antibody tests have an important role to play in these cases, as they provide an assessment of both short-term and long-term trends of antibody response, as well as antibody abundance and diversity. Consequently, these tests are of vital importance for dental professionals. Endodontists being on the front line to address dental emergencies are consequently at a higher risk than other health workers since most procedures involve aerosol generation. Understanding the disease process and the various testing modalities will help to ensure safety of the clinician while ensuring adequate patient care.

KEYWORDS: RT-PCR, ELISA, Rapid antigen test, Antibody response, Dental treatment, Chest CT.

INTRODUCTION

A lot of research has been conducted and published highlighting the implications of COVID-19 in clinical dental care. Most of it recommends that elective dental treatment maybe deferred and clinicians may focus on emergency care for the time being. Yu et al. (2020) studied the characteristics of dental emergencies during the COVID-19 epidemic in Wuhan, China. They found that only dental emergency cases were referred to the hospital during online health consultations. The authors also concluded that the majority (50.6%) of the dental emergencies were of endodontic origin in a COVID-19 affected area. Endodontists are on the front line to address such a crisis and to relieve the distressed patients of pain.[1, 2] Consequently, they are at a higher risk than other health workers since most procedures result in aerosol generation. Therefore, through this article we aim to bring to light the various diagnostic and investigating modalities along with its implications in dental practice.

Diagnosis

COVID-19 is known to exhibit a wide range of clinical symptoms; from mild flu-like symptoms to severe respiratory distress and therefore warrants an efficient testing system. Identification of COVID-19 patients at early stages will allow prompt intervention for patients with life-threatening complications.

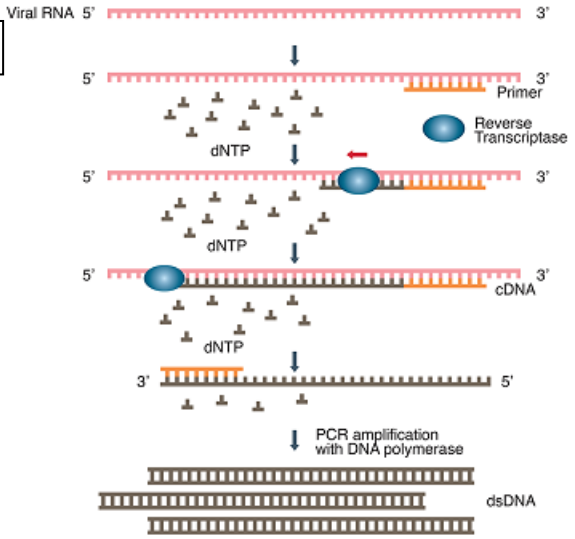
Commercially available testing modalities fall into two broad categories: The first category includes molecular assays for detection of SARS-CoV-2 viral RNA using polymerase chain reaction (PCR)-based techniques or nucleic acid hybridization-related strategies. Viral RNA testing identifies infected individuals during the acute phase of infection. Serological and immunological assays are included in the second category and they largely depend on detection of antibodies as a result of exposure to the virus or on detection of antigenic proteins in infected individuals. This technique identifies individuals who have developed antibodies to the virus and could be potential convalescent plasma donors. It also improves

the ability to conduct contact tracing and monitor the immune status of individuals and groups over time.[3]

Molecular assays for detection of viral nucleic acid

A) Reverse transcription-polymerase chain reaction: RT-PCR (Image 1)[3] is based on its ability to amplify a tiny amount of viral genetic material in a sample and is considered to be the gold standard for identification of SARS-CoV-2 virus. Currently, RT-PCR tests for COVID-19 generally use samples collected from the upper respiratory system using swabs. RT-PCR assay (TaqPath COVID-19 Combo kit) that uses self-collected saliva samples is also available nowadays.[4, 5, 6] RT-PCR begins with laboratory conversion of viral RNA into DNA by RNA-dependent DNA polymerase (reverse transcriptase). This reaction relies on small DNA sequence primers designed to specifically recognize complementary sequences on the RNA viral genome and the reverse transcriptase to generate a short complementary DNA copy (cDNA) of the viral RNA. In real-time RT-PCR, the amplification of DNA is monitored in real time as the PCR reaction progresses. This is done using a fluorescent dye or a sequence-specific labeled DNA probe. An automated system then repeats the amplification process for about 40 cycles until the viral cDNA can be detected, usually by a fluorescent or electrical signal.[7] Traditionally, this test is carried out as one-step or a two-step procedure. One-step real-time RT-PCR uses a single tube containing the necessary primers to run the entire RT-PCR reaction. Two-step real-time RT-PCR involves more than one tube to run the separate reverse transcription and amplification reactions, but offers greater flexibility and higher sensitivity than the one-step procedure. It requires less starting material and allows for the ability to stock cDNA for quantification of multiple targets[8] The one-step procedure is generally the preferred approach for detection of SARS-CoV-2 because it is quick to set up and involves limited sample handling and reduced bench time, decreasing chances for pipetting errors and cross-contamination between the RT and real-time PCR steps. To date, the majority of molecular diagnostic tests have utilized the real-time RT-PCR technology targeting different SARS-CoV-2 genomic regions.[9, 10, 11, 12] Even though RT-PCR is the most widely used method of testing, it has the disadvantage of requiring expensive laboratory instrumentation highly skilled laboratory personnel and can take days to generate results. RT-PCR tests are constantly evolving with improved methods and automated procedures.

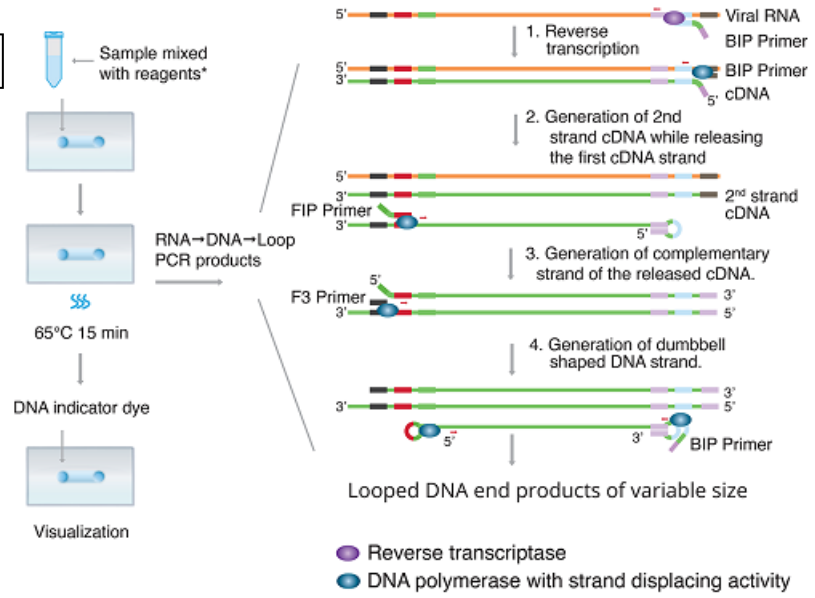
Image 1



B) Isothermal nucleic acid amplification: RT-PCR requires multiple temperature changes for each cycle, involving sophisticated thermal cycling equipment.[13] Isothermal nucleic acid amplification is an alternative strategy that allows amplification at a constant temperature and eliminates the need for a thermal cycler.

a) Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP) has been developed as a rapid and cost-effective alternative for SARS-CoV-2. RT-LAMP (Image 2)[3] requires a set of four primers specific for the target region to enhance the sensitivity and combines LAMP with a reverse transcription step to allow for the detection of RNA. The amplification product is detected via photometry which measures the turbidity caused by magnesium pyrophosphate precipitate in solution as a byproduct of amplification. The reaction can be followed in real time either by measuring the turbidity or by fluorescence using intercalating dyes. Real-time RT-LAMP testing has promising simplicity and sensitivity as it requires only heating and visual inspection.[14] It is also a rapid technique and requires 13 minutes or less but is restricted to one sample per run.[15, 16]

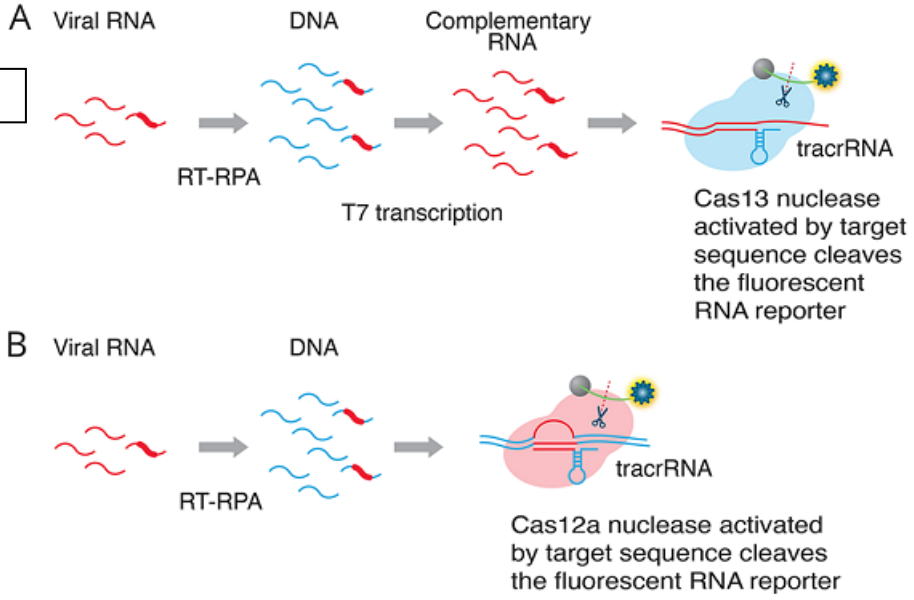
Image 2



b) Transcription-mediated amplification (TMA) is a patented, single tube, amplificati on technology. It is carried out under isothermal conditions and is adopted from retroviral replication. It can be used to amplify specific regions of either RNA or DNA with a better efficiency as compared to RT-PCR.[17] The retroviral reverse transcriptase and T7 RNA Polymerase utilized in this technique is used for detection of nucleic acids from pathogens. The testing kit by Hologic Inc called Panther Fusion system works on this principle and can perform both RT-PCR and TMA. Its high testing output (up to 10000 tests in 24h) is its chief advantage while simultaneously evaluating for other viruses with overlapping symptoms.[18]

c) Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) represents a family of nucleic acid sequences found in prokaryotic organisms like bacteria. These sequences can be recognized and cut by a set of bacterial enzymes, called CRISPR-associated enzymes, represented by Cas9, Cas12 or Cas13. Certain enzymes in the Cas12 and Cas13 families can be programmed to target and cut viral RNA sequences followed by isothermal amplification of the target, resulting in a visual readout with a fluorophore.[19] CRISPR-based assays do not require complex instrumentation and can be read using paper strips to detect the presence of the SARS-CoV-2 virus. These tests are low-cost and may be performed in as little as 1 h, without loss of sensitivity or specificity.[20, 21, 22] The illustration in Image 3[3], A is SHERLOCK assay and B is DETECTR assay.

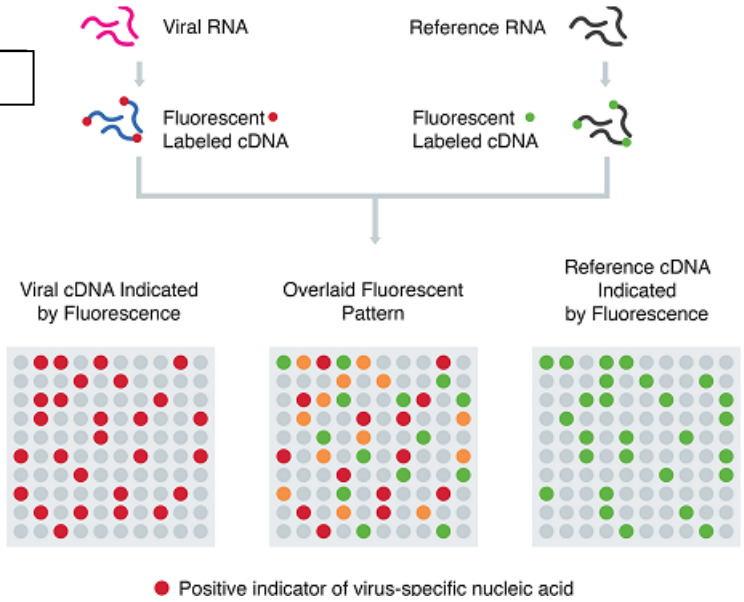
Image 3



d) Rolling Circle amplification (RCA) is capable of 10^9-fold signal amplification of each circle within 90 min. RCA is advantageous in that it can be performed under isothermal conditions with minimal reagents and avoids the generation of false-positive results frequently encountered in PCR-based assays. An efficient assay for the detection of SARS CoV by RCA was previously performed in both liquid and solid phases and used to test clinical respiratory specimens. In clinical scenario, this technique is not in use presently.[23]

C) Nucleic acid hybridization using microarray: It is a rapid high-output nucleic acid detection modality. It is based on generation of cDNA from viral RNA using reverse transcription followed by labeling of cDNA with specific probes. The labeled cDNAs are loaded into the wells of microarray trays containing solid-phase oligonucleotides fixed onto their surfaces. If they hybridize (Image 4)[3], they will remain bound after washing away the unbound DNA, thus indicating the presence of virus-specific nucleic acid.[24] The microarray assay has proven useful in identifying mutations associated with SARS-CoV in spike (S) gene with absolute accuracy.[25]

Image 4



D) Amplicon-Based Metagenomic sequencing: It relies on dual approach including amplicon-based sequencing and metagenomic sequencing. The advantage of metagenomic sequencing is regarding rapid identification of secondary pathogens which may be contributing in severity of COVID-19 symptoms. Dual technique is particularly relevant to SARS-CoV-2 in estimation of its rate of mutation and to identify its possible recombination with other human coronaviruses. In addition to potential contact tracing, molecular epidemiology, and studies of viral evolution; metagenomic approaches such as sequence-independent single primer amplification (SISPA) provide additional checks on sequence divergence. Altogether they have implications for vaccine development and antiviral efficacy.[26]

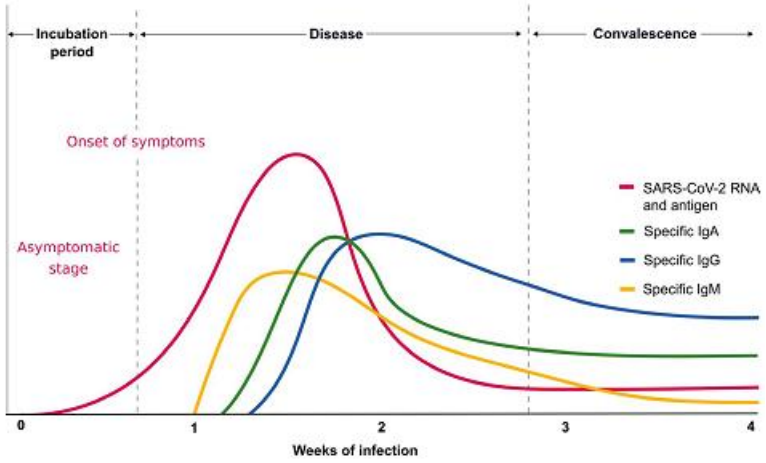
From about 112 currently available molecular assays for detecting SARS-CoV-2, 90% utilize PCR or RT-PCR technologies, 6% utilize isothermal amplification technologies, 2% utilize hybridization technologies and 2% utilize CRISPR-based technologies.

Serological and immunological assays

Serological testing is defined as an analysis of blood serum or plasma and has been operationally expanded to include testing of saliva, sputum, and other biological fluids for the presence of immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies. RT-PCR-based viral RNA detection, while used widely; cannot be used to monitor the progress of the disease stages and cannot be applied to broad identification of past infection and immunity. That is where these tests play an important role, providing an assessment of both short-term (days to weeks) and long-term (years or permanence) trends of antibody response, as well as antibody abundance and diversity. Consequently, they are vital in epidemiology and vaccine development.

Based on a normal antibody response (Image 5);[27] an early rise of IgM antibody followed by IgG is expected. In SARS-CoV-2, IgM antibody response starts and peaks within 7 days; continues till the acute phase of the disease lasts. IgG antibodies develop several days after IgM and continue as protective antibodies lifelong. Thus, IgM may be considered as an indicator of early stage infection and IgG can be an indicator of current or prior infection. IgG may also be used to suggest the presence of post-infection immunity.[21, 28, 29, 30]

Image 5



According to Long QX et al, specific IgG levels in serum could already be raised against SARS-CoV-2 at the same time as IgM or earlier.[31] According to Lee YL et al, virus-specific IgG and IgM against SARS-CoV-2, reached to peak levels 17-19 days and 20-22 days after symptom onset, respectively. Patients with severe manifestations had higher antibody titers when compared with non-severe patients. Various patterns of seroconversion reported were, synchronous seroconversion of IgG and IgM, IgM seroconversion earlier than IgG and IgM seroconversion later than IgG.[32] To KK et al utilized enzyme immunoassay in their study for analyzing specific IgM and IgG. More patients were found seropositive for IgG than IgM at day 0 and day 5 of hospital admission and greater proportion of patients in that study also had earlier IgG seroconversion.[33] SARS-CoV-2 serum IgA appears as early as two days after onset of symptoms and possibly earlier than IgG or IgM. In consistence with this finding, early appearance of mucosal IgA has been reported. It is the principal component of mucosal immunity and can easily be measured in saliva. On one hand, low levels of sIgA have been associated with an increased incidence of respiratory infections; sIgA can induce neutrophil activation resulting in an inflammatory response in the airway. Although, no sIgA (secretory IgA) tests for SARS-CoV-2 have been authorized till date, research is desirable to establish correlations between clinical outcome and excess sIgA levels.[34, 35, 36]

Table I - Antibody testing in symptomatic patients and its correlation in dental practice.

SYMPTOMATIC [37, 38]			
Test result	**Interpretation**	**Measures**	**Dental implication**
Both IgG & IgM Negative	• >7 days – Disease is less likely to be COVID-19 • <7 days – Not yet reflected in the tests	• >7 days – Practice social distancing • <7 days - Isolation recommended. Repeat testing after 7 days of symptom onset	• >7 days – Can undergo treatment with precautions • <7 days – Assesment after 7 days of symptom onset
IgG positive, IgM negative	Subject is likely in the later stages of the disease but may still be contagious.	Remain isolated for 14 days.	Defer treatments for 14 days following which evaluation maybe done.
IgM positive, IgG negative	Subject is actively producing antibodies to a recent infection. Subject is contagious and may spread disease	Immediately isolate for atleast 14 days. Repeat testing after 14-21 days to determine IgG status before returning to normal activities	Defer treatments for up to 3-5 days after symptoms subside or up to 21-35 days from symptom onset.
Both IgG, IgM positive	Subject's immune system is actively producing antibodies to an ongoing infection which began more than 14 days ago	Isolate immediately for atleast 14 days. Repeat testing after 7-14 days to confirm IgG only status before returning to normal activities.	Defer treatment for up to 3-5 days after symptoms subside or up to 21-35 days from symptom onset.

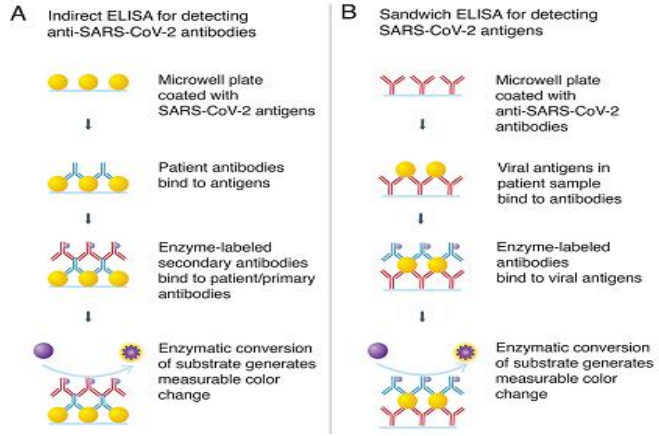
Table II - Antibody testing in symptomatic patients and its correlation in dental practice.

ASYMPTOMATIC[37, 38]			
Test result	**Interpretation**	**Measures**	**Dental implication**
Both IgG & IgM Negative	Subject is not suspected of COVID-19 infection and it is less likely that subject has had infection in the past. However this does not rule out recent exposure.	Practice social distancing.	Patient can be treated by following protocol for aerosol and non-aerosol procedures
IgG positive, IgM negative	Likely had infection several weeks ago. Some degree of functional immunity is present and it is unlikely that the subject is contagious.	Practice social distancing.	Patient can be treated by following protocol for aerosol and non-aerosol procedures
IgM positive, IgG negative	Subject is actively producing antibodies to a recent infection. Subject is contagious and may spread disease	Immediately isolate for atleast 14 days. Repeat testing after 14-21 days to determine IgG status before returning to normal activities	Defer treatments for up to 3-5 days after symptoms subside or up to 21-35 days from symptom onset.
Both IgG, IgM positive	Subject's immune system is actively producing antibodies to an ongoing infection which began more than 14 days ago	Isolate immediately for atleast 14 days. Repeat testing after 7-14 days to confirm IgG only status before returning to normal activities.	Defer treatment for up to 3-5 days after symptoms subside or up to 21-35 days from symptom onset.

The determination of SARS-CoV-2 exposure relies largely on the detection of either IgM or IgG antibodies that are specific for various viral antigens including, but not exclusively, the spike glycoprotein and nucleocapsid protein. Various modalities for these determinations include the traditional enzyme-linked immunosorbent assay (ELISA), immunochromatographic lateral flow assay, neutralization bioassay, and specific chemosensors. While advantages like speed, multiplexing and automation have made these techniques popular; disadvantages like requirement of trained personnel and dedicated laboratories limit its use. An alternative to these antibody testing methods are the rapid antigen tests wherein antibodies are used to identify presence of viral antigens in serological samples.[23]

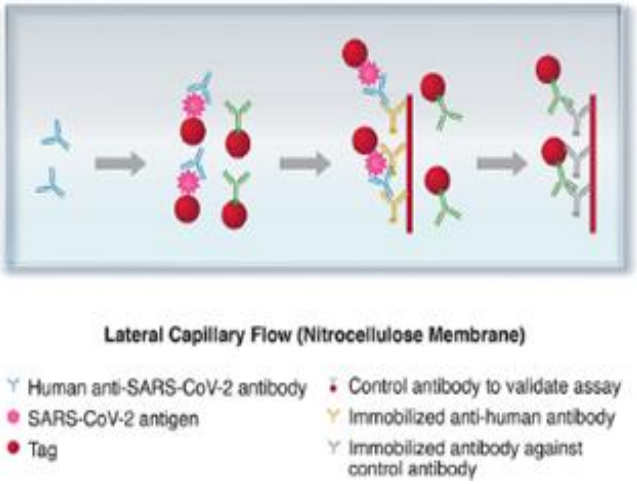
a) Enzyme-linked immunosorbent assay (ELISA): ELISA (Image 6)[3] is designed for quantifying substances like proteins, peptides, hormones and antibodies using a microwell, plate-based method. It requires 1-5hours and can give both qualitative and quantitative results. The wells are coated with a viral protein. The antiviral antibodies if present in the patient samples will bind specifically, and the bound antibody-protein complex can be detected with an additional tracer antibody to produce a colorimetric or fluorescent-based readout. ELISA is speedy, has the ability to test multiple samples and is adaptable to automation for increased throughput but can be variable in sensitivity.

Image 6



b) Lateral flow immunoassay (Image 7)[3]**:** This small, portable, qualitative (positive or negative) chromatographic assay is a type of rapid diagnostic test (RDT) where results are obtained in 10-30 minutes. Fluid samples are applied to a substrate material that allows the sample to flow past a band of immobilized viral antigen. If present, anti-CoV antibodies are collected at the band, where, along with co-collected tracer antibodies, a color develops to indicate the results. The test is inexpensive and does not require trained personnel.[3]

Image 7



c) Neutralization assay: This method determines the ability of an antibody to inhibit virus infection of cultured cells. Patient samples of whole blood, serum, or plasma are diluted and added to cell cultures at decreasing concentrations. If neutralizing antibodies are present, their levels can be measured by determining the threshold at which they are able to prevent viral replication in the infected cell cultures. The time to results for neutralization assays is typically 3-5 days, but recent advances have reduced this to hours. This technique requires cell culture facilities and in the case of SARS coronavirus, Biosafety Level 3 (BSL3) laboratories are indispensible. Despite these limitations, determination of neutralizing antibodies is important in the short term for the therapeutic application of convalescent plasma and in the long term, for vaccine development.[39, 40]

d) Luminescent immunoassay: They involve chemiluminescence and fluorescence. They are fully automated tests and run on chemiluminescence analyzer.[41]

e) Biosensor test: Biosensor tests rely on converting the specific interaction of biomolecules into a measurable readout via optical, electrical, enzymatic, and other methods. Surface plasmon resonance (SPR) is a technique that measures interference with incident light at a solid boundary due to local disturbances such as the adsorption of antibody or antigen.[42] An SPR-based biosensor was developed for the diagnosis of SARS using coronaviral surface antigen (SCVme) anchored onto a gold substrate. It is expected to be available for research May 2020 onwards.[43]

f) Rapid antigen test: Based on specific monoclonal antibodies to provide a mechanism to capture viral antigens from the sample. These tests allow detection of viral antigens and are complementary to the molecular genetic assays.[44, 45, 46] They are available in variety of formats like colorimetric enzyme immunoassay for SARS-CoV, enhanced chemiluminescent immunoassay for SARS-CoV and fluorescence lateral flow assay for detection of SARS-CoV-2 nucleocapsid protein.[47, 48]

Most of these tests are still in developmental stages and their use has been limited. A strong limiting factor is that only a small subset of patients with positive molecular assays is seropositive, owing to the delay in production of antibodies. Stronger evidence is required indicating correlation between seropositivity with immune protection.[3]

Chest ct scans

Chest CT is a conventional, noninvasive imaging modality with high accuracy and speed. On the basis of available data published in recent literature, almost all patients with COVID-19 had characteristic CT features in the disease process, such as different degrees of ground-glass opacities with and/or without crazy-paving sign, multifocal organizing pneumonia, and architectural distortion in a peripheral distribution. Compared with reverse-transcription polymerase chain reaction (RT-PCR), chest CT may be a more reliable, practical and rapid method to diagnose and assess COVID-19, especially in the area affected by the epidemic. According to Tao Ai et al, with RT-PCR results as the reference standard in 1014 patients, the sensitivity, specificity, and accuracy of chest CT in indicating COVID-19 infection were 97% (580 of 601 patients), 25% (105 of 413 patients), and 68% (685 of 1014 patients), respectively. The positive predictive value and

negative predictive value were 65% (580 of 888 patients) and 83% (105 of 126 patients), respectively.

In their study, additionally, about 60% of patients (34 of 57) had typical CT features consistent with COVID-19 before (or parallel to) the initial positive RT-PCR results, and almost all patients (56 of 57) had initial positive chest CT scans before or within 6 days of the initial positive RT-PCR results. This indicates that CT can be very useful in the early detection of suspected cases.[49]

The urgent need for accurate and rapid diagnosis of SARS-CoV-2 infection remains critical as global healthcare systems continue to operate during the course of the COVID-19 pandemic. While RT-PCR has been the dominant technique for detection of viral RNA, other nucleic acid assays including isothermal amplification assays, hybridization microarray assays, amplicon-based metagenomics sequencing, and the cutting-edge CRISPR-related technologies are also under development or have resulted in approved tests. Significant progress has been made in the development of diagnostic tests despite all the remaining questions and challenges. Ongoing global efforts are working to communicate and facilitate new diagnostic solutions to promote more accurate and faster identification of cases. Extensive research is being conducted in the course of the pandemic and there is constant update in literature regarding the same. Fresh perspectives may be unveiled and may lead to novel discoveries. Therefore, clinical implications of the above mentioned data should be carried out discretely.

In conclusion, tests can be an effective tool to mitigate risks for patients and healthcare workers. Further research should be conducted to validate rapid serological tests as they are cheap and rapid. This can represent a great chance for restarting dentistry in a sustainable manner.

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