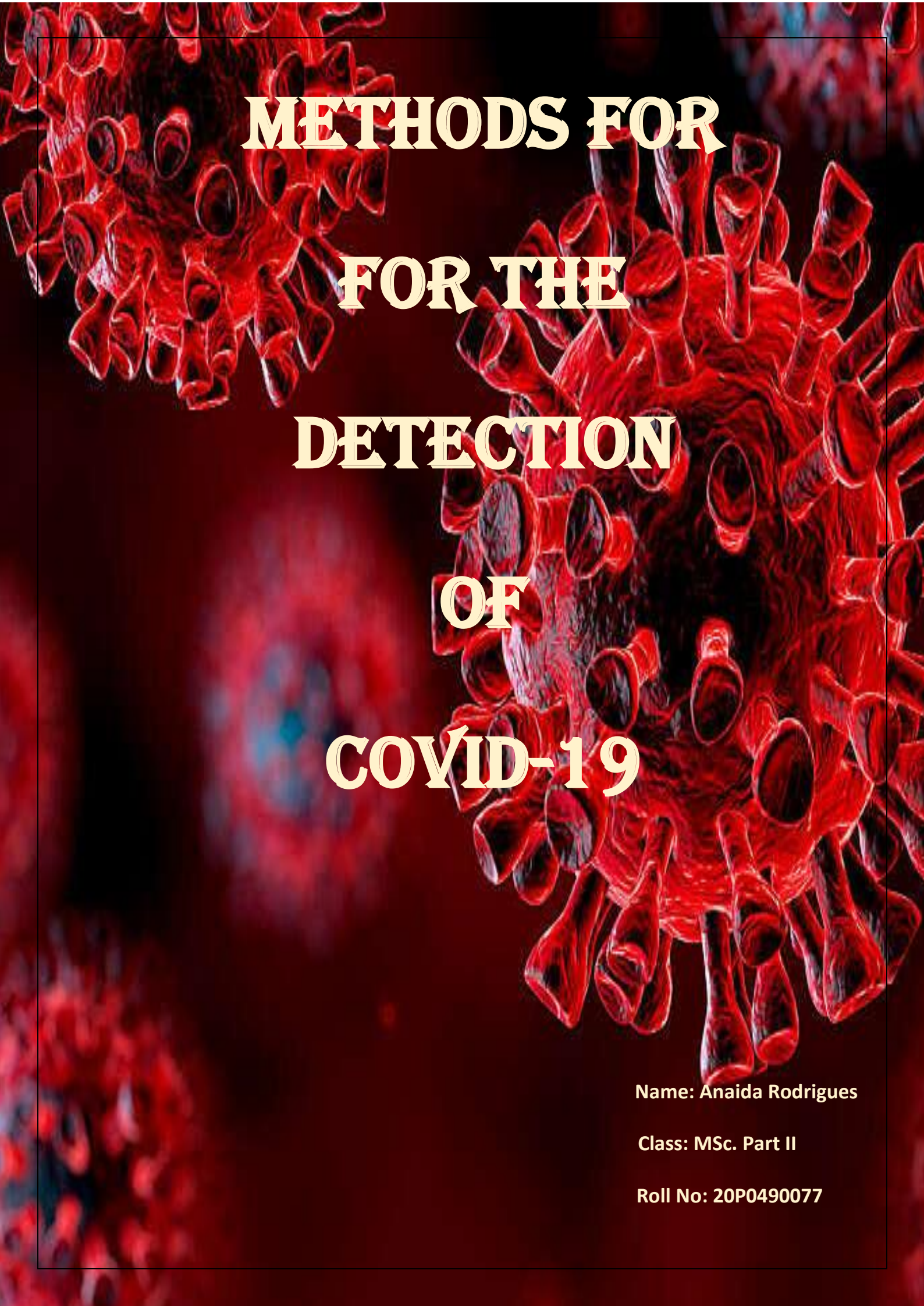

METHODS FOR FOR THE DETECTION OF COVID-19

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CERTIFICATE

This is to certify that the dissertation entitled '**Methods for the Detection of COVID-19'** is bonafide work carried out by Ms. Anaida Rodrigues under my supervision in partial fulfilment of the requirement for the award of the degree of Master of Science in Chemistry at the Department of Chemistry, Goa University.

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METHODS FOR THE DETECTION OF COVID-19

A DISSERTATION REPORT

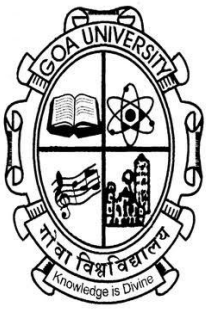
Submitted in Partial Fulfilment

Of

The degree of M. Sc. (Analytical Chemistry)

By

Ms. Anaida Rodrigues



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APRIL 2022

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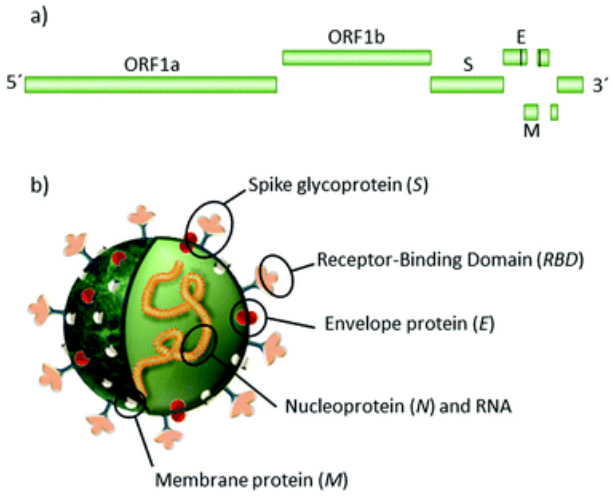
Abstract

Coronavirus disease 2019 (COVID-19) is a newly emerging human infectious disease caused by severe acute respiratory syndrome coronavirus-2. Based on the rapid increase in the rate of human infection the World Health Organisation (WHO) has classified the COVID-19 as a pandemic on March 12, 2020. Extensive efforts in testing for Coronavirus infection combined with isolating infected cases and quarantining those in contact, use of masks and frequent hand washing have proven successful in bringing the epidemic under control. One of the pivotal interventions to control SARS-CoV-2 transmission is its early detection. This study presents an overview on the methods for the detection of COVID-19

Introduction

Viruses are microscopic entities that have a core of genetic material, either DNA or RNA. The core is covered with a capsid, a protective coat made of protein. Around the capsid, there may be a spiky covering known as the envelope. These spikes are proteins that enable viruses to bind to and enter host cells. There, if the conditions are right, they can multiply. They do not contain ribosomes, so they cannot make proteins. This makes them unable to reproduce independently and totally dependent on their host. After entering a host cell, a virus hijacks the cell by releasing its own genetic material and proteins into the host. It uses the host cellular machinery to make many copies of itself.

Some viruses cause disease for example, the severe acute respiratory syndrome coronavirus 2, or SARS-CoV-2 causes the disease called COVID-19 due to which the world is presently experiencing an unprecedented health crisis. The first cases were reported in December 2019 in Wuhan, China. Then it rapidly spread worldwide in only a few days. Coronaviruses can be classified into 4 genera: α, β, γ and δ.[1] These viruses are detected in a wide range of animal species, including humans. Human alphacoronaviruses, 229E, and NL63 and betacoronaviruses, OC43 and HKU1, SARS and MERS coronaviruses.[2] This is the seventh corona virus to infect human. Two other notable examples include severe acute respiratory syndrome and Middle East respiratory syndrome, the former of which began in southern China and resulted in 774 deaths in 8098 infected individuals in 29 countries from November 2002 through July 2003, and the latter of which originated in Saudi Arabia and was responsible for 848 deaths among 2458 individuals in 27 countries through July 2019.



The SARS-COV-2 belongs to the genus β coronavirus which is comprised of crown-like, enveloped, positive single stranded RNA viruses with a diameter of 80-90nm. The genome is organised into 3 main regions: two open reading frames (ORF), ORF1a and ORF1b, located at the 5′ end. The third region comprises genes encoding the structural and accessory proteins at the 3′ end (fig a). It consists of four structural proteins named as S (spike), E (envelope), M (membrane) and (nucleocapsid). The N protein holds the RNA genome of the virus and S, E and M proteins create the virus envelope together (fig b). SARS-CoV-2 have been found to use the receptor angiotensin converting enzyme 2 (ACE2) for entry into host cells. The receptor binding domain region (RBD) of the SP is the major target for SARS-CoV-2 therapy and detection. [3]

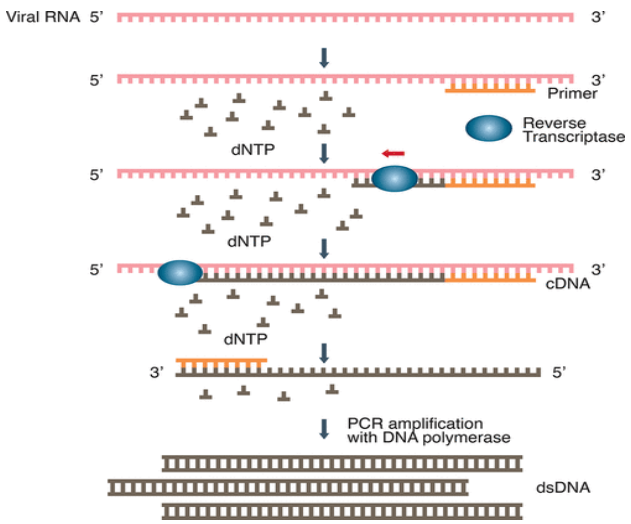
The virus is transmitted from infected individuals to others who are in close contact from droplets expelled from the respiratory tract or through contact with contaminated surfaces. The symptoms of COVID-19 patients range from relatively mild illness, such as fever, a sore throat and cough, fatigue, aches, pains to shortness of breath and fatal pneumonia. On average, the human incubation period is 5-6 days, but it can extend upto-14 days. The virus is particularly deadly to the young and elderly.

A number of covid-19 identification methods based on the detection of virus nucleic acids, virus proteins or specific antibodies generated by immune system have been developed. Two types of sample specimens used for the diagnosis of covid-19 are respiratory samples for direct detection of virus and serum samples for identification of antiviral antibodies. Detection of SARS-COV-2 in patient sample is a critical tool for monitoring spread of the disease, guiding therapeutic decision and devising social distancing protocols. This review is intended to be an overview on the methods for the detection of COVID-19.

Methods used for the detection of COVID-19

1. Detection of COVID-19 based RT-PCR

Reverse transcription polymerase chain reaction (RT-PCR) is a nucleic acid-based detection process which directly detects the viral nucleic acid in the specimens collected. It is the most widely used method for the detection of SARS-CoV-2 nucleic acid using respiratory samples. RT-PCR starts with laboratory conversion of viral genomic RNA into DNA by RNA dependent DNA polymerase (reverse transcriptase).[4] This conversion is done because some viruses such as the SARS-CoV-2 coronavirus which causes COVID-19 only contain RNA, which means they rely on infiltrating healthy cells to multiply and survive. Once inside the cell, the virus uses its own genetic code -RNA in the case of COVID-19 virus to take control of and 'reprogramme' the cells, turning them into virus-making factories. This reaction relies on small DNA sequence primers designed to specifically recognise complimentary sequences on the RNA viral genome and the reverse transcriptase to generate short complementary (cDNA) of the viral RNA. (fig. a). 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 sequence specific DNA probe labelled with a fluorescent molecule and quencher molecule. 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.



(Fig. a) Reverse transcription polymerase chain reaction (RT-PCR)[4]

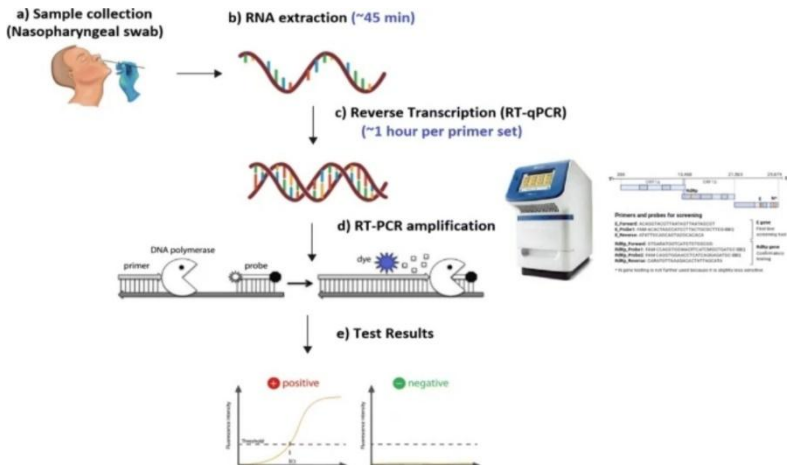
Corman *et al.*[5] aligned and analysed a number of SARS-related viral genome sequence to design a set of primers and probes. (fig. b). Among the SARS related viral genomes three regions that had conserved sequences were discovered: 1) the RdRP gene - RNA dependent RNA polymerase gene/ in the open reading frame ORF1ab region. 2) the E gene (envelope protein gene) and 3) the N gene (nucleocapsid protein gene).

Assay/use	Oligonucleotide	Sequence[a]	Concentration[b]
RdRP gene	RdRp_SARSr-F	GTGARATGGTCATGTGTGGCGG	Use 600 nM per reaction
	RdRp_SARSr-P2	FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ	Specific for 2019-nCoV, will not detect SARS-CoV. Use 100 nM per reaction and mix with P1
	RdRP_SARSr-P1	FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ	Pan Sarbeco-Probe will detect 2019-nCoV, SARS-CoV and bat-SARS-related CoVs. Use 100 nM per reaction and mix with P2
	RdRp_SARSr-R	CARATGTTAAASACACTATTAGCATA	Use 800 nM per reaction
E gene	E_Sarbeco_F	ACAGGTACGTTAATAGTTAATAGCGT	Use 400 nM per reaction
	E_Sarbeco_P1	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ	Use 200 nM per reaction
	E_Sarbeco_R	ATATTGCAGCAGTACGCACACA	Use 400 nM per reaction
N gene	N_Sarbeco_F	CACATTGGCACCCGCAATC	Use 600 nM per reaction
	N_Sarbeco_P	FAM-ACTTCCTCAAGGAACAACATTGCCA-BBQ	Use 200 nM per reaction
	N_Sarbeco_R	GAGGAACGAGAAGAGGCTTG	Use 800 nM per reaction

(fig. b) Primers and probes used for real-time RT-PCR[5]

RT-PCR is carried out in one step or two step procedures. The one step procedure is faster which uses a single tube containing the necessary primers to run the entire RT-PCR reaction. The two-step procedure involves more than one tube to run the separate reverse transcription and amplification reactions offers greater flexibility, higher sensitivity requires less starting material and allows for the ability to stock cDNA for quantification of multiple targets than one step procedure. One step approach is generally preferred as it has advantages of limited sample handling, reduced batch time, decreased chances for pipetting errors and cross contamination between RT and real time PCR steps.[4]

The United State Centres for Disease control and Prevention (CDC) uses one step real time RT-PCR (rRT-PCR) assay, which provides quantitative information on viral loads, to detect the presence of SARS-C0V-2. To perform the assay, the viral RNA is extracted and added to a master mix which contains nuclease free water, forward and reverse primers, a fluorophore quencher probe and a reaction mix consisting of reverse transcriptase, polymerase, magnesium, nucleotides and additives. The master mix and extracted RNA are loaded into a PCR thermocycler and the incubation temperatures are set to run the assay. During rRT-PCR, the fluorophore quencher probe is cleaved, generating a fluorescent signal. And the amplified progress is recorded in real time. (fig. c). The reaction takes ~41 minutes and occur in 96-well plate where each well contains a different sample or control.

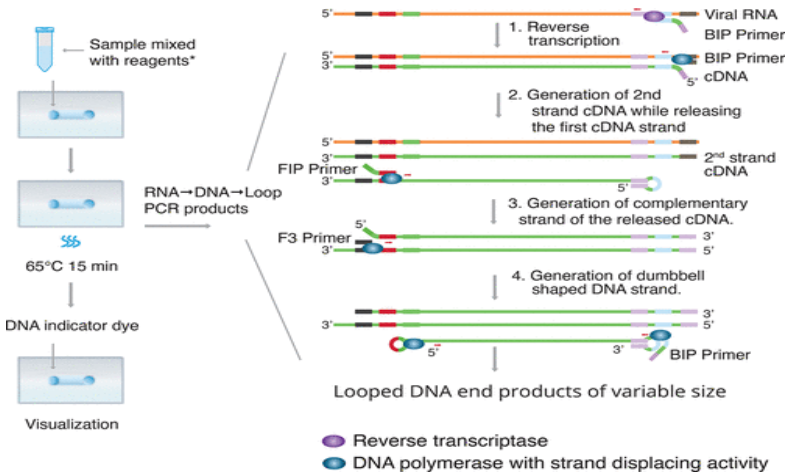


(fig. c)

RT-PCR test takes less than hour to couple of days to give results. The sensitivity of these method usually depends on the RNA amount in each sample. Although RT-PCR is currently the gold standard for SARS-CoV-2 detection due to its capacity to directly measure the viral genomic parts rather than the secondary biomarkers such as antigens or antibodies there are still some limitations: only a limited number of tests can be performed each day, relatively time consuming, requires skilled personnel and its application is limited in countries that lack sufficient resources to handle large scale testing. Improper sampling operations, low viral load in the sample collection or at the collection time, nonstandard transportation process and viral mutation have led to negative results. It does not give information for the patients who have already recovered, since the viral load is cleared out of the body after recovery.

2. Detection of COVID-19 based on Loop Mediated Isothermal Amplification method

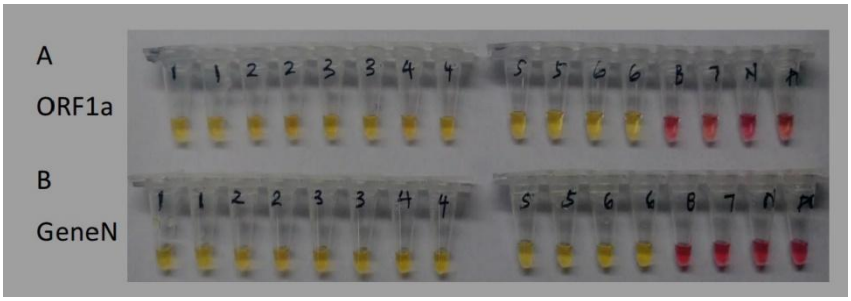
Isothermal amplification techniques are conducted at a constant temperature of 60-65°C and do not need specialised laboratory equipment to provide similar analytical sensitivities to polymerase chain reaction (PCR). Loop mediated isothermal amplification was developed by Notomi *et al*. in 2000. This method has been widely applied for the detection of influenza virus, middle east - respiratory syndrome-CoV,[6] west Nile virus,[7] Ebola virus,[8] Zika virus,[9] yellow fever virus and a variety of other pathogens. These techniques include recombinase polymerase amplification, helicase-dependent amplification, and loop mediated isothermal amplification (LAMP).



(fig. a) Reverse transcription loop mediated isothermal amplification[4]

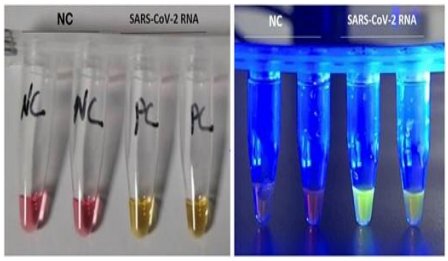
Reverse transcription loop-mediated isothermal amplification (RT-LAMP) uses DNA polymerase and four to six primers to bind to six distinct regions of the target genome. (fig. a). In a four-primer system, there are two inner primers a forward and a reverse inner primer and two outer primers. In LAMP diagnostic tests, a patient sample is added to the test tube and the amplified DNA is detected by turbidity caused by magnesium pyrophosphate in solution, a by-product of the reaction, by change in colour with addition of pH sensitive dyes such as SYBR Green,[10] phenol red, calcein, by fluorescence based on fluorophores that recognise and bind to dsDNA in real time or by immunochromatography.

Zhang *et al*. [11] reported the successful amplification of LAMP methodology to identify COVID-19 viral RNA from purified RNA or patient cell lysis using a visual colorimetric method using pH sensitive dyes to visualise the LAMP amplification via the change in the pH resulting in from proton accumulation due to incorporation of deoxynucleoside triphosphate (dNTP's). These results were further verified using RNA sample purified from respiratory swabs collected from COVID-19 patients in Wuhan, China. In addition to controls, 7 patient's RNA samples were tested, 6 samples tested positive by RT-qPCR using ORF1a primers and 4 positives with gene N primers. One sample was negative by both RT-qPCR sets. When these samples were tested in the colorimetric LAMP assay all 6 RT-qPCR positive sample showed visible colour change indicating positive amplification, while the single RT-qPCR negative sample maintained pink colour and was judged negative. (fig. b). Thus, the results were 100% in agreement with those from the qRT-PCR method. A sensitivity/LOD as low as 4.8 copies/ µl was achieved. This colorimetric LAMP provides simple, rapid method for SARS-C0V-2 detection. Not only purified RNA can be used as sample input, but also direct tissue or cell lysate may be used without an RNA purification step.



(fig. b). Yellow indicates positive detection after 30 minutes of incubation for sample (1-6) and pink a negative reaction for sample (7) with results compared to negative control (N) [11]

Yu *et al.*[12] described a LAMP method named iLACO (isothermal LAMP) method for the rapid detection of SARS-CoV-2. The method was validated on the RNA sample extracted from SARS-CoV-2; RT-qPCR verified positive patients. A colour change from pink to light yellow in the reaction tubes after 20 minutes of incubation at 65°C was observed. (fig. c). iLACO could detect as low as 10 copies per µl copies of ORF1ab gene. SYBR Green dye was added into the reaction mixture for expansion of iLACO detection capability and the colour change was checked with a Gel imaging system. GeneFinder™ a new type of nucleic acid dye enhanced the fluorescence signal and sensitivity. By exposure under blue light, green fluorescence was observed with naked eye in positive reaction with 100 copies per µl, whereas it remained pink in the negative control.



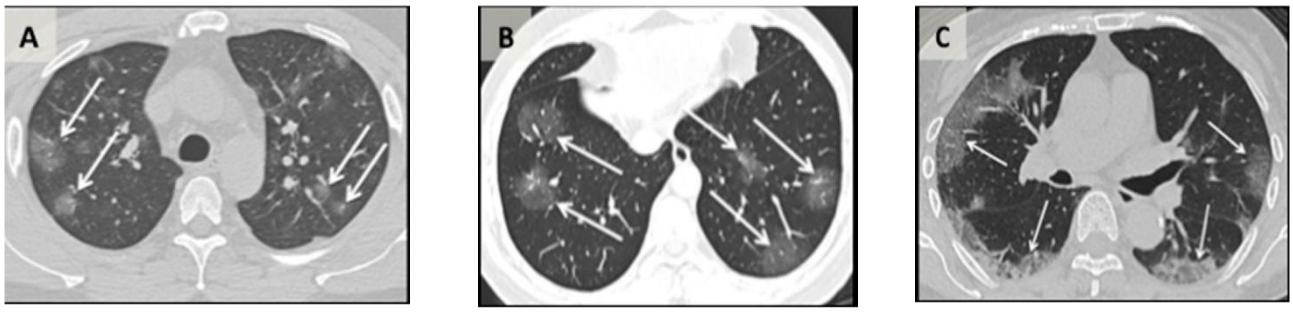
(fig. c). Fluorescent signal detected by UV and blue light for iLACO. Positive signal was visible with the naked eye with colorimetric pH indicator or under blue light with GeneFinder™ dye. NC and PC refers to the negative and COVID-19 positive controls respectively. [12]

RT-LAMP has several advantages as the reaction occurs in <1 hour at 60-65°C, the reagent used are relatively cheap and stable at room temperature and therefore holds a promise for use outside of a central laboratory by staff without specialised training and without need of advanced equipment like thermocycler. This method is simple to operate and easy to visualize for detection. The drawbacks to LAMP are the challenges of optimizing primers and reaction conditions.

3. Detection of COVID-19 based on Computed Tomography (CT)

Computed tomography is commonly referred to as a CT scan. A CT scan is a diagnostic imaging procedure that uses a combination of X-rays and computer technology to produce images of the inside of the body, including the bones, muscles, fat, organs and blood vessels.

Due to shortage of kits and false negative rate of RT-PCR, the Hubei Province, China temporarily used CT scans as clinical diagnosis for COVID-19. Chest CT scans are non-invasive and involve taking many X-ray measurements at different angles across a patient's chest to produce cross-sectional images. The images are analysed by radiologists to look for abnormal features that can lead to a diagnosis. The imaging features of C0VID-19 are diverse and depend on the stage of infection after the onset of symptoms. For example, Bernheim *et al*.[13] characterized chest CT findings in patients with COVID-19 in China in relationship to the time between symptom onset and the initial CT scan i.e., 0-2 days (36 patients), intermediate 3-5 days (33 patients), late 6-12 days (25 patients). The most hallmark features of COVID-19 on images were bilateral and peripheral ground-glass opacities (increase of hazy opacity) and consolidative pulmonary opacities (fluid or solid materials in compressible lung tissue). Notably, 20 of the 36 patients (56%) imaged in the early phase had a normal CT scan. With longer time after the onset of symptoms, CT findings were more frequent including consolidation, bilateral and peripheral disease, greater total lung involvement, linear opacities, crazy paving patterns and the reverse halo sign. Bilateral lung involvement was observed in 10 of the 36 early patients (25%), 25 of 33 intermediate patients (76%) and 22 of the 25 late patients (88%).

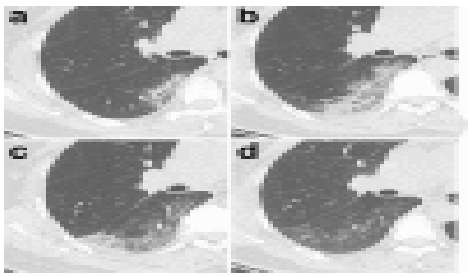


Axial CT image obtained without intravenous contrast material. The arrows indicating:

- **A.** Bilateral ground glass opacities in upper lobes in a 36-year-old man
- **B.** Larger ground opacities in the bilateral lower lobes with a rounded morphology in 44-year-old man
- **C.** Bilateral ground glass and consolidative opacities with a striking peripheral distribution.

Axial CT image obtained without intravenous contrast material in a 43-year-old woman showing **A)** crazy paving pattern as manifested by right lower lobe ground glass opacification with interlobular septal thickening with intralobular lines **B)** axial CT image obtained in a 22-year-old woman showing an area of faint ground glass opacification in left upper lobe with a ring of denser consolidation (arrow, reverse halo sign)

Pan *et al* [14] reported the cases who recovered from pneumonia due to COVID-19. They identified that there are four stages based on chest CT results. The first stage is the early stage (0-4 days) that showed a small ground glass opacities (GGO) distributed subpleural in the lower part. The second stage is the progressive stage (5-8 days), with infection quickly extended to a bilateral multi-lobe with diffused GGO, consolidation, and crazy paving pattern. The third stage is the peak stage (10-13 days) that showed a slow expansion of the involved part of the peak involvement, including diffused GGO, crazy-paving pattern, residual parenchymal bands, and consolidation. Whereas the final stage is the absorption stage, which occurred two weeks after the onset of the first symptoms, which showed that the disease was managed, and the consolidation was slowly absorbed.



Typical evolution of CT findings in a 47-year female patient presenting with persistent fever (38°C) for three days. **a)** at presentation day, a small region of subpleural GGO with partial consolidation was demonstrated in the right lower lobe; **b)** day 7, there was an enlarged region of GGO with superimposed inter- and intralobular septal thickening with partial consolidation; **c)** day 11, partial resolution of the initial GGO, with a new area of subpleural consolidation; **d)** day 20, continued resolution with minimal residual GGO and parenchymal bands were observed

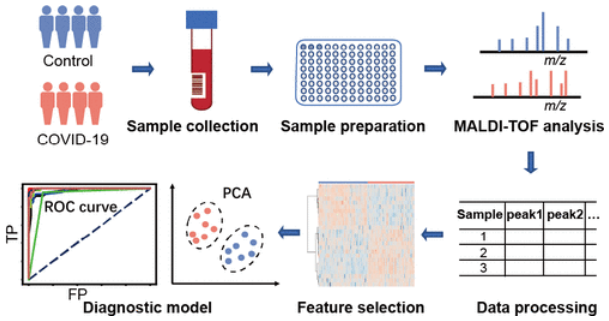
Unfortunately, CT systems are expensive, requires technical expertise and it is limited to large central hospitals. Images from chest CT scan is not specific and is only related to the spots in the lungs that may be caused by the virus. Therefore, this information needs clinical symptoms to validate that patient is truly positive for COVID-19. The main caveat of using CT for COVID-19 is that the specificity is low (25%) because the imaging features overlap with the other viral pneumonia.

4. Detection of COVID-19 based on Mass Spectrometry

Detection using Matrix Assisted laser desorption ionisation- time of flight (MALDI-TOF)

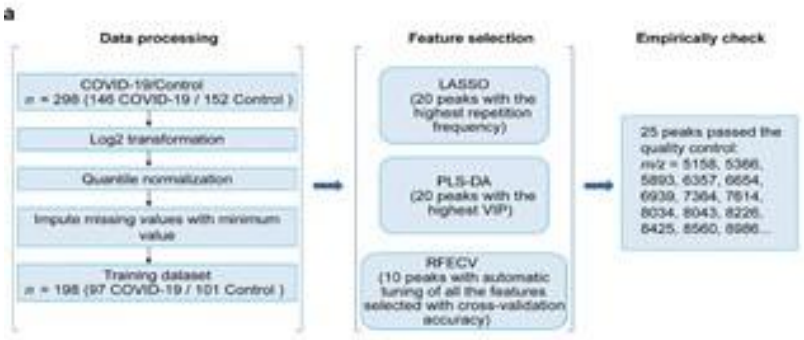
MALDI is a soft ionisation technique used in Mass spectrometry that can preserve the structure of large, non-volatile and labile molecules, allowing for the sensitive detection of many kinds of non-volatile biomolecules including proteins, nucleic acids and carbohydrates.

Yan *et al*. [15] developed a high throughput serum peptidome profiling method based on matrix assisted laser desorption/ionisation time of flight mass spectrometry (scheme 1) for rapid detection of COVID-19. Serum samples collected from COVID-19 patients were analysed with MALDI-TOF after sample pre-treatment.



SCHEME 1

After MS data processing and feature selection according to the process (scheme 2) ,25 MS peaks had shown distinctive features between COVID-19 patients and control participants. Classification models for the detection of covid-19 were built on 8 machine learning methods. All the models achieved area under the curve (AUC) above 0.99. A logistic regression method showed highest accuracy of (99%) with sensitivity 98% and specificity of 100% for the 25 feature peaks.



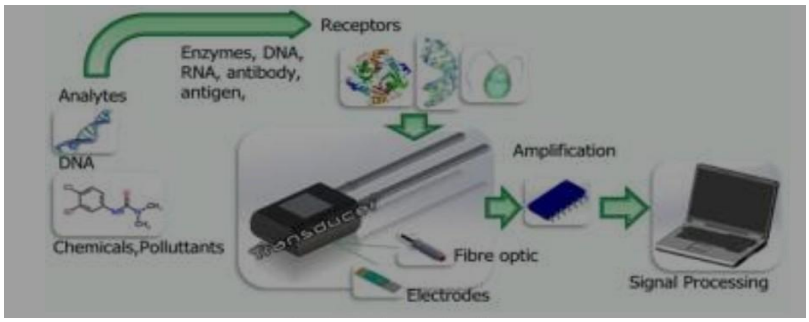
SCHEME 2

This MALDI-TOF MS based serum peptidome profiling method does not need an extra clean testing environment and has a low risk of sample cross contamination. Moreover, the per analysis requires only 5 micro litre of serum sample and can be analysed immediately after sterilization. It takes less than 1 minute for analysis and without use of any expensive consumables, the cost per test by MALDI-TOF MS is much lower compared to PCR or immunoassay.

Similarly, Nachtigall *et al* [16] also detected SARS-COV-2 in nasal swabs instead of serum peptidome using MALDI-TOF analysis

5. Detection of COVID-19 based on biosensors

Sensors consist of chemical or biological receptors and transducers. The receptor interacts specifically with a target analyte and the transducer converts the recognition process into a quantitative signal. Biosensors are analytical devices in which biological recognition molecules such as enzymes, antibodies, or nucleic acids are coupled with a transducer and a detector that detects the interacted analyte and gives a digital output. (fig. a).[17]

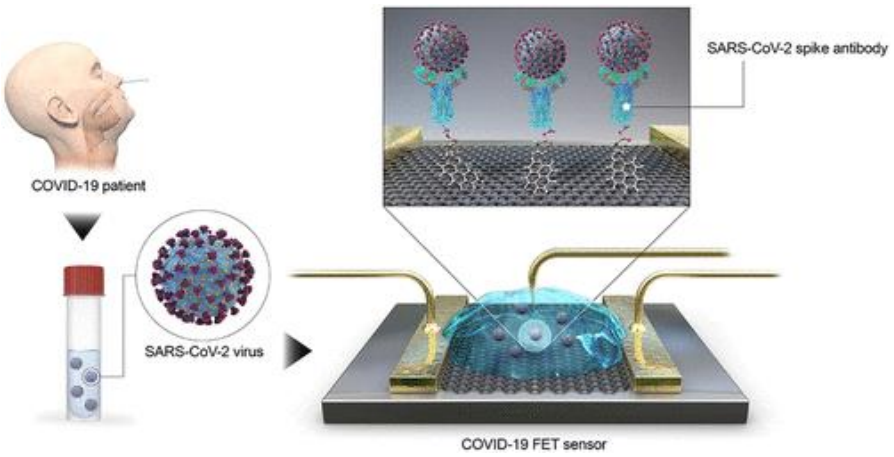


(fig. a). Working of biosensors[17]

- **Graphene Field Effect Transistor (FET) based biosensor**

FET biosensor are based on the interaction of specific antibodies with a specific antigen (SARS-CoV-2). Antigen detect the binding of antibodies to the antigen by immobilizing the reaction on the surface of a transducer that converts the surface change parameters into detectable electrical signal. These variations in voltage and current is created by the resistance developed on the surface of the bio receptors, which is further processed by signal processing unit and gives final result. Graphene-based FET biosensors can detect surrounding changes on their surface and provide an optimal sensing environment for ultrasensitive and low noise detection.

Graphene, 2D hexagonally arranged carbon based single atom thick layer has proven its worth towards the advanced biosensing platforms. This is mainly because of numerous exceptional properties such as high specific area, high electrical, and ionic mobility. Thus, by exploiting these properties of graphene, Gaurav *et* al.[17] developed a graphene bio-FET- based biosensor to detect the SARS-CoV-2 virus. (fig. b)

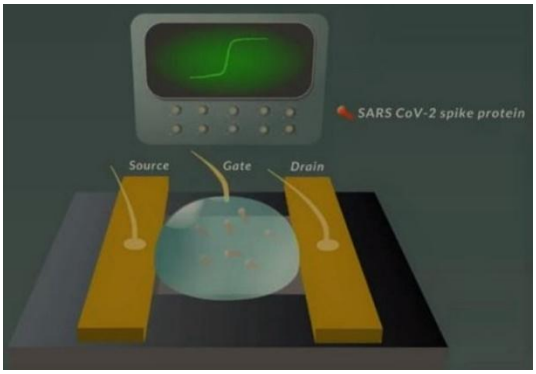


(fig. b). COVID-19 FET sensor operation procedure[16]

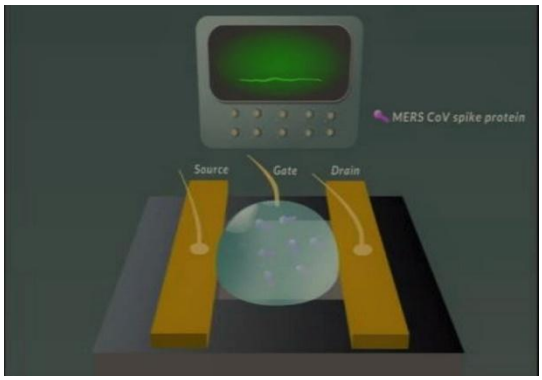
In these study, graphene sheets of FET were coated with a specific antibody to detect SARS-CoV-2 spike protein. This protein was chosen because of being a major transmembrane protein of the virus and highly immunogenic. The FET- based biosensing platform was developed by using a conventional wet transfer method, graphene was coated on the SiO_2/Si substrate. Next a coating of poly (methyl methacrylate) (PMMA) C4 was applied to the graphene layer. After transferring the PMMA/ graphene layer to the SiO_2/Si substrate, the PMMA layer was removed using acetone. A gold-chromium electrode layer was later fabricated on the etched graphene layer. For the

immobilization of the SARS-COV-2 antibody on the graphene layer, first it was immersed in 2Mm 1-pyrenebutyric acid N-hydroxy succinimide ester (PBASE), is a linker that contains a pyrene group that non-covalently attaches to the graphene through $\pi - \pi$ stacking, at the other end is the activated ester which reacts with the amines, then adding SARS-CoV-2 spike antibody it reacts with the linker to form chemical bond.

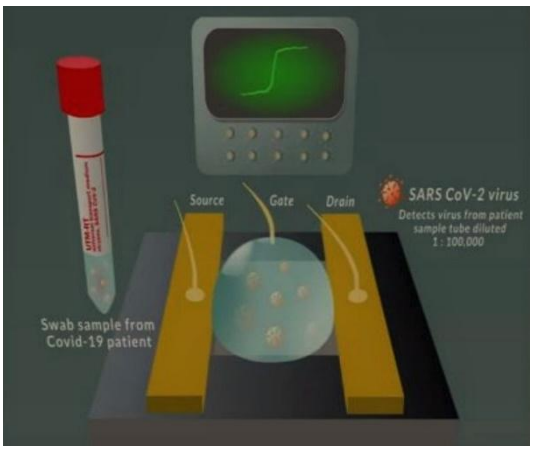
The sensitivity of this biosensor was tested after the graphene was attached to the antibody. The binding of spike protein to the antibody alters the distribution in the vicinity of the graphene layer, and therefore, changes its electrical conductivity (fig. a). Consequently, the current flowing between the source and drain electrodes changes and the biosensor is able to detect the spike protein in clinical samples with the limit of detection of 1fg/ml. The sensor showed no response for the spike proteins of other viruses viz. MERS-CoV virus (fig. b). The actual swab sample collected from a COVID-19 patient placed on the G-FET could detect the virus. The higher the concentration of the SARS-CoV-2 virus in the sample, the greater response was seen. (fig. c). One of these biosensor features was distinguishing between the non-infected and infected people with SARS-CoV-2 virus.

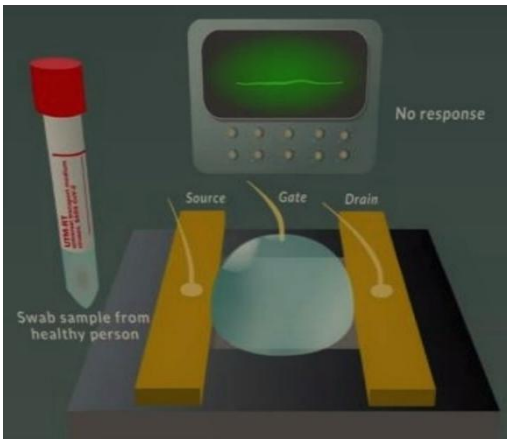


(Fig.a)



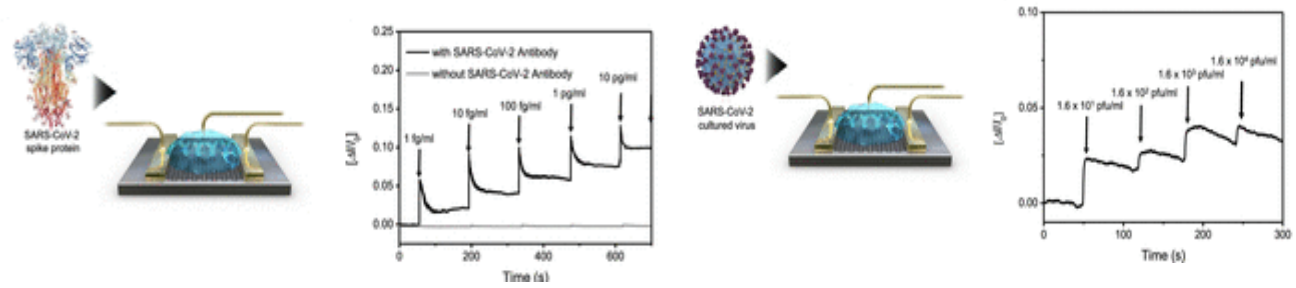
(Fig.b)



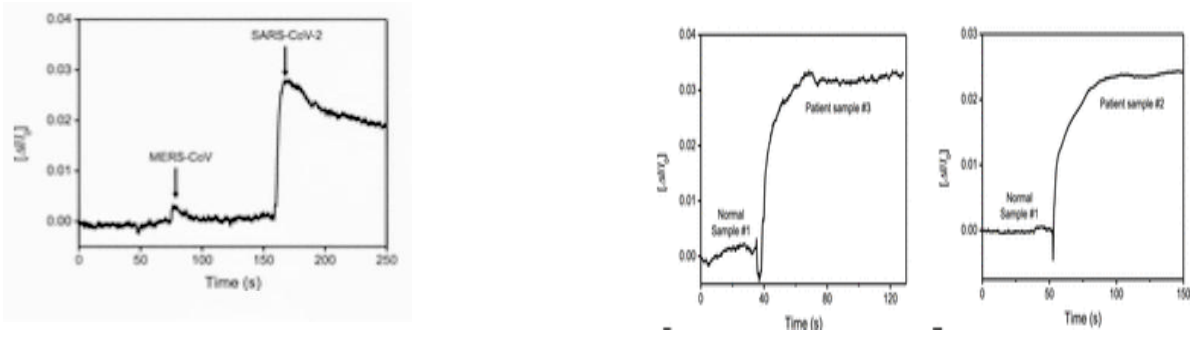


(Fig .c) (fig. d)

Similarly, Seo *et al*.[18] also developed graphene-based biosensor fabricated with SARS-CoV-2 spike antibodies. The graphene layer was passivated with a photoresist and chemically functionalized with PBASE. The total sensing area was a dimension of 100×100 μm^2. After the successful binding of PBASE with the graphene layer, two different peaks were observed after X-ray photoelectron spectroscopy as a result of the relative resonance of sp^3 bonding. To detect the SARS-CoV-2, phosphate buffer saline was added as an electrolyte to create an aqueous gated biosensor. With the help of the Current-Voltage graph there is variation in current. After the attachment of the coronavirus spike protein to the detecting surface changes in current was observed. The nasopharyngeal swab samples of COVID-19 patients, antigens, and the cultured virus were used to access this FET- based biosensor's performance. Detection of SARS-CoV-2 spike protein was measured at the concentration of 1fg/ml in phosphate -buffered saline whereas 100fg/ml in case of the universal transport medium. This graphene-based FET sensor exhibited LOD, 1.6×10^1 pfu/ml, and 242 copies/ml in case of SARS-CoV-2 spiked and clinical samples. Furthermore, when response to related but distinct spike protein from the MERS-CoV was tested the sensor showed no response to the spike protein, establishing that the FET- based biosensor was highly specific for the SARS-COV-2 spike antigen protein. Moreover, this biosensor was able to detect SARS-COV-2 spike protein in clinical samples without any preparation.



A B



C D

Real time-response of COVID-19 FET sensor for detection of SARS-CoV-2 towards **(A)**- antigen protein, **(B**)- Cultured virus, **(C)**- Target SARS-CoV-2 antigen protein and MERS-CoV protein, **(D)**- COVID-19 patients.

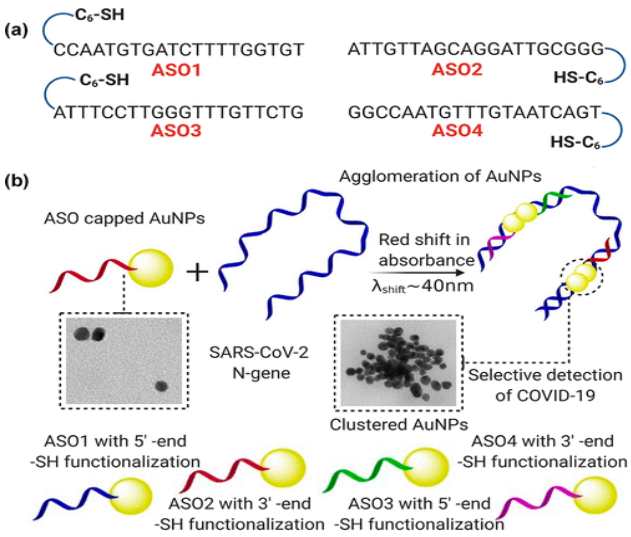
These results demonstrated that FET-based sensor platform provides simple, rapid and highly responsive detection of SARS-CoV-2 virus in clinical samples. However, the need for purified and high-quality graphene is difficult to be obtained at kilogram or industrial scale for making a large number of such devices. Since the purity of graphene is one of the major concerned needed for these devices to have higher sensitivity, producing graphene with consistent quality is still a challenge and any error in the making process can lead to inaccurate biosensor.

- **Colorimetric sensors**

Colorimetric sensors are detectors that give a noticeable change in colour in the visible range (380-700 nm) when in contact with any analyte. They are associated with the class of optical sensors. Colorimetric sensors consist of a receptor, transducer, and a detecting domain, but the layout and the choice of the material depend on the type of external stimuli interacting with it. The optical properties of nanoparticle are linked with its surface plasmon resonance (SPR). When there is any optical polarization, the electric field of the NP is enhanced, resulting in light scattering and absorption at a specific frequency of SPR. This adjustable surface plasmon resonance acquired by the nanoparticle helps them to change colour used in the functioning of nanobiosensors. For example, when AuNPs of 20 nm are suspended in an aqueous solution, they give a typical red wine colour (~530nm) due to the SPR peak in the absorption spectrum. But when these nanoparticles agglomerate the colour changes owing to the change in the plasmon resonance.

Moitra *et al*. [19] developed a colorimetric biosensing approach for the naked eye detection of SARS-CoV-2 with the aid of Au plasmonic nanoparticles. Plasmonic nanoparticles work as a detection tool when the frequency of the photon incident on it matches the frequency of the excited electrons of the nanoparticles. This change in SPR peak causes a change that does not require any detection machinery, and thus, the results can be interpreted even by a layman. These AuNPs, when capped with suitably designed thiol modified antisense oligonucleotides (ASOs) specific for N- gene (nucleocapsid phosphoprotein) of SARS-CoV-2, could potentially be used for diagnosing positive COVID-19 cases within 10 minutes. For the working of this sensor, four ASO sequences covering two regions of the viral genome sequence, that is N- gene of SARS-Cov-2, at the same time were designed. These ASOs were capped on plasmonic nanoparticles after thiolating them at

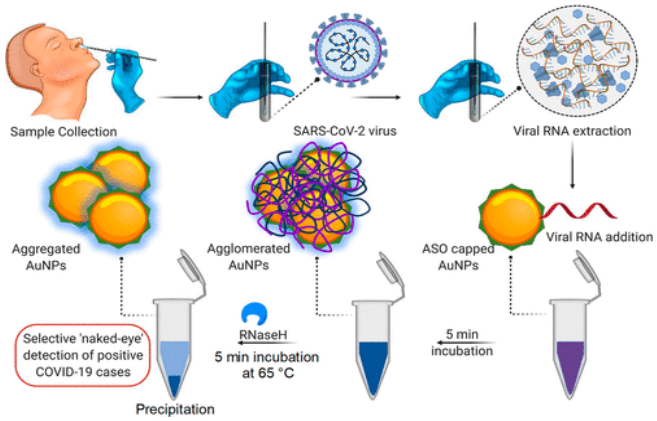
one end. Since these ASO capped NPs showed agglomeration only in the presence of the SARS-Cov-2 RNA sequence, the evident change in colour with the specific binding could show positive results. ASO1 and ASO3 were thiolated at their 5'end while ASO2 and ASO were thiolate at their 3'end.



(a) representation of differentially functionalised ASOs with their sequences.

(b) The proposed concept behind the agglomeration of gold nanoparticles when capped with the ASOs, is schematically presented in (b).

The hydrodynamic diameter of the NPs was significantly increased when SARS-CoV-2 RNA was attached to it, which amplified its tendency of aggregation. A red shift of 40 nm was observed with an increase in the absorbance at the wavelength of 600 nm. The change in the SPR peak changed the colour of the solution from violet to dark blue. This change involves a minor red shift of 40nm. Further it was found that the addition of RNaseH (non – sequence specific endonuclease) cleaves the RNA strand from the composite hybrid of RNA and Au-ASOmix, leading to a visually detectable precipitate from the solution mediated by the additional agglomeration among the AuNPs. (fig. c). The sensitivity of this COVID-19 biosensor has also been tested against MERS-CoV viral RNA where no distinct change in absorbance was found with MERS-CoV RNA.



(fig.c) Selective Naked eye detection of SARS-CoV-2 RNA by the sutably designed ASO-capped AuNPs[19]

This colorimetric assay provides reliable, reproducible, selective, and visual naked eye detection of COVID-19 causative virus, SARS-CoV-2 which is devoid of the requirement of any sophisticated instrumental techniques. In addition to this, the current methodology ensures its feasibility even with the mutated N gene forms of the virus during its spread as the assay has been designed to simultaneously target two separate regions of the gene.

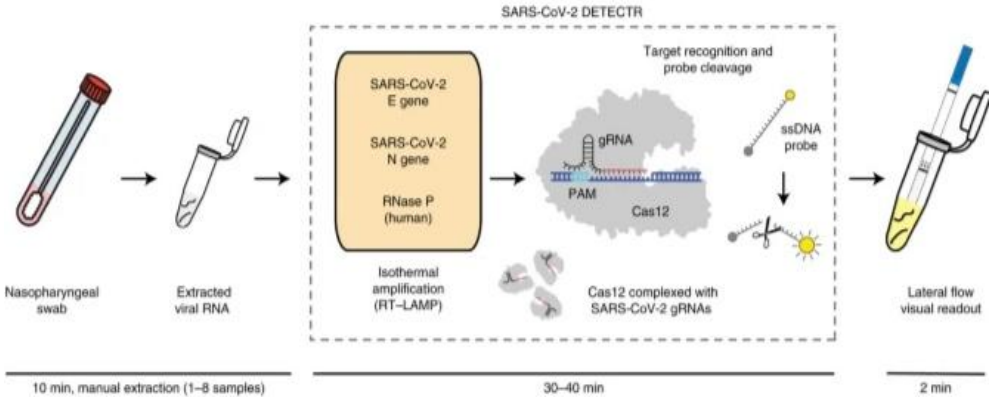
6. Detection of Covid-19 based on CRISPR-Cas method

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) represents a family of nucleic acid sequence found in prokaryotic organisms, such as bacteria.[3] These sequences can be recognised and cut by a set of bacterial enzymes, called CRISPR- associated enzymes, exemplified by Cas9, Cas12 and Cas13. CRISPR-Cas system had been used for treating HIV patients. Although it did not cure the patient in the first attempt, the therapy was found to be safe as it did not cause any adverse events during the 19 months follow up. In addition, the CRISPR-Cas system is also used for the treatment of cancer and blindness.

The principle relies on the endonuclease activity of CRISPR-Cas enzymes that recognise the presence of specific sequences via programmable CRISPR-RNA (CrRNA). When the CrRNA binds to target sequence, the endonuclease activity is triggered and the recognised nucleic acid is cut. For diagnostic purposes, Cas12 and Cas13a are preferred enzymes, as they present a secondary activity of nonspecific cutting once the specific endonuclease activity has been triggered. Cas12 targets dsDNA and its secondary activity determines the multiple cuts of any ssRNA found. Cas 13a targets ssRNA and its secondary activity determines the cutting of cis and trans ssRNA.[3] The use of

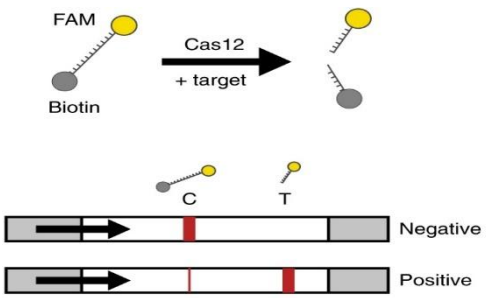
linked quenched/fluorescent probes permits the detection of a fluorescent signal after the endonuclease activity cleaves the reporter molecule, indicating the presence of nucleic acid.

Broughton *et al.* [20] have adopted this molecular test to detect SARS-CoV-2 in RNA extracted samples in <40 minutes. The test is called SARS-CoV-2 DNA endonuclease target CRISPR trans reporter (DETECTR), and is based on RT-LAMP amplification at 62°C for 20-30 minutes, followed by the detection of predefined coronavirus sequence by the enzyme Cas12. (fig. a). The primers target the E and N genes and human RNase P for quality control.



(fig. a). SARS-CoV-2 DETECTR workflow

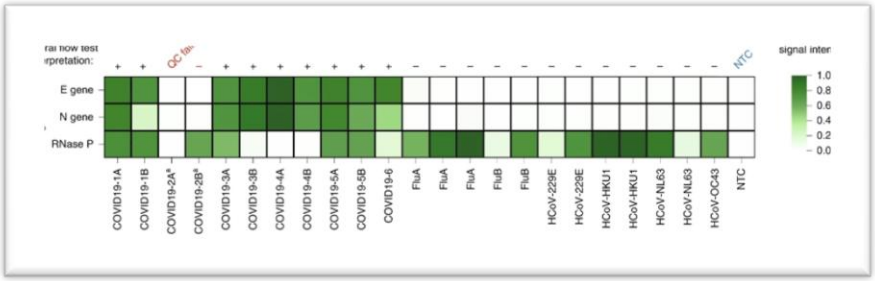
The SARS-CoV-2 DETECTR assay is considered positive if there is detection of both E and N genes or presumptive positive if there is detection of either E or N gene. Visualisation of the Cas12 detection reaction is achieved using fluorescence and lateral flow strips, using 6-carboxy-fluorescein-biotin conjugate as the receptor molecule that binds to the control line if uncleaved, and to the detection line when cleaved by Cas12. (fig. b).



(fig. b). DETECTR coupled with lateral flow readout[19]

Clinical samples from six patients with covid-19 infection (n=11, 5 replicates) and 12 patients infected with influenza or one the four seasonal coronaviruses (HCoV-229E, HCoV-HKU1, HCoV-

NL63, HCoV-OC43) (N=12) were analysed using SARS-CoV-2 DETECTR assay in which SARS-CoV-2 was detected in 9 of 11 swabs and did not show cross reactivity with other respiratory viruses. (fig. c). The estimated LOD for the DETECTR assay was 10 copies per µl reaction. The sensitivity and specificity relative to the CDC qRt-PCR based assay were 95% and 100% respectively for the detection of coronavirus in 80 total respiratory swab samples.

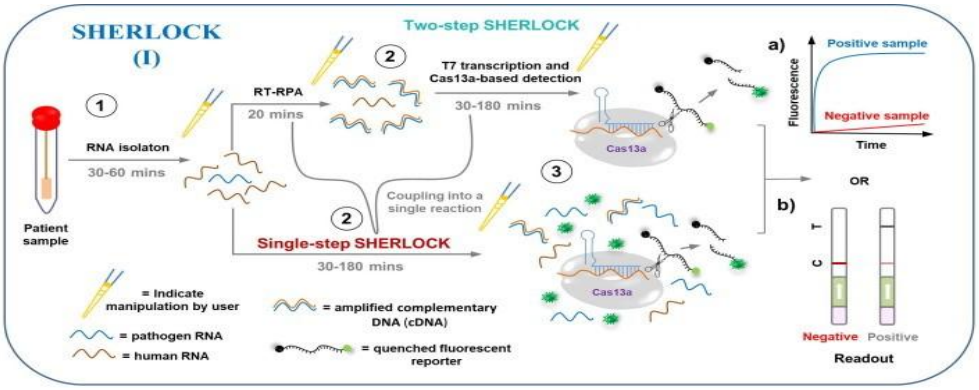


(fig. c)**.** Patient sample DETECTR data from lateral flow readout[20]

As the DETECTR assay uses similar sample collection and RNA extraction methods as the CDC assay and other qRT-PCR assays it is subject to the same potential limitations with regard to the availability of personal protective equipment, extraction kits and reagents. However, its key advantages over qRT-PCR include isothermal signal amplification obviating the need for thermocycling, rapid turnaround time, single nucleotide target specificity, integration with accessible and easy to use reporting formats such as lateral flow strips and no requirement for complex laboratory infrastructure.

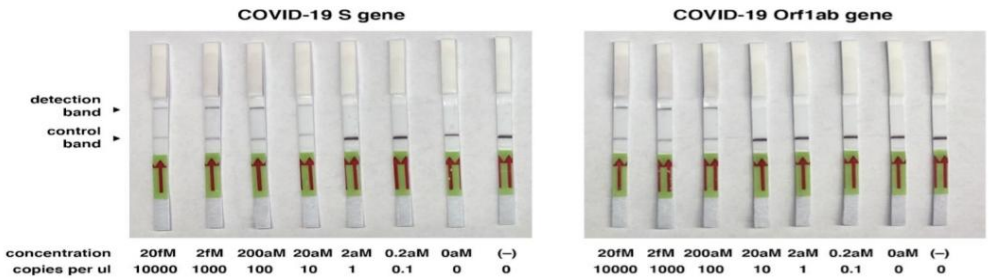
Another CRISPR based detection system **SHERLOCK-** Specific High Sensitivity Enzymatic Reporter UnLOCKing that targets S and ORF1ab gene fragments of SARS-CoV-2 was described by SHERLOCK bioscience which uses Cas13a ribonuclease for RNA sensing.

SHERLOCK starts with an isothermal target nucleic acid preamplification step, in which either a DNA or an RNA target input is replicated by loop-mediated isothermal amplification (LAMP), most often using recombinase polymerase amplification (RPA). Subsequently, the amplified targets are converted to RNA via T7 transcription. The specificity of Cas13 is conferred by crRNA- target pairing, and further sensitivity is achieved through signal amplification by the collateral cleavage activity of Cas13 on the RNA reporters that are added to the reaction, which can be captured on a colorimetric lateral flow strip by biotin fluorescein RNA reporters or visualised by fluorescence signal. (Scheme 1)



(Scheme 1). Workflow of SHERLOCK based detection of SARS-CoV-2

Zhang *et al.*[21] described the SARS-CoV-2/SHERLOCK protocol to detect the novel coronavirus, by employing synthetic SARS-CoV-2 strains that are available in databases. SHERLOCK/SARS-CoV-2 showed high sensitivity in detecting both S gene, which encodes the spike (S) proteins of the virus, and the Orf1ab gene, which encodes Orf1ab polyproteins. Positive COVID-19 result is indicated by the appearance of 2 lines for both S and Orf1ab genes using lateral flow strip. (fig. d). The limit of detection for this protocol was between 10-100 SARS-CoV-2 RNA copies per microliter of input, within first 40 minutes of amplification.



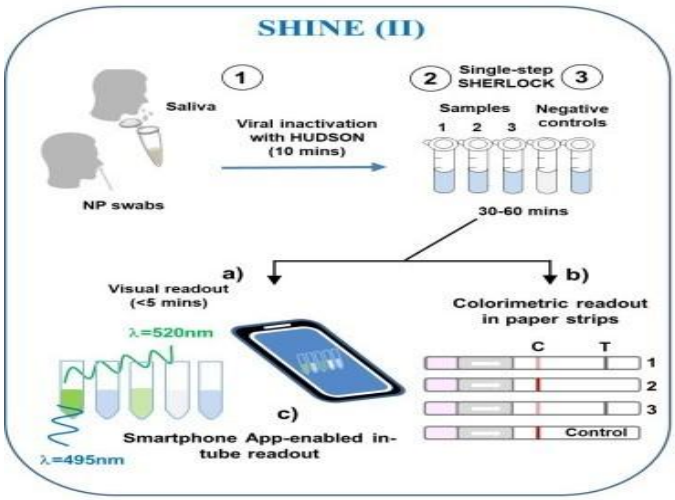
(fig. d). Lateral flow readout for different input concentration.[21]

The advantages of CRISPR-Cas based SHERLOCK method include rapid turnaround time, limited infrastructure requirements and isothermal amplification that avoids the need for thermocycling.

- **SHINE**

During the current outbreak, there is unprecedented demand for the reagents and sophisticated equipment necessary for SARS-CoV-2 detection. In this regard, Myhrvold *et al*.[22] previously established an approach to release viral nucleic acids from clinical specimens directly and to protect them from degradation, thus bypassing the need for nucleic acid extraction. This method named HUDSON (Heating Unextracted Diagnostic Samples to Obliterate Nucleases), employs heat and chemical reduction to inactivate the pervasive ribonucleases found in body fluids. Then viral

particles are lysed by disruption of the viral envelope, resulting in the release of nucleic acids into solution. HUDSON-treated biological samples can be directly added to isothermal amplification reaction mixtures without inhibiting subsequent amplification or detection. Therefore, some lab environment and professionally trained personnel are not essential for this protocol.



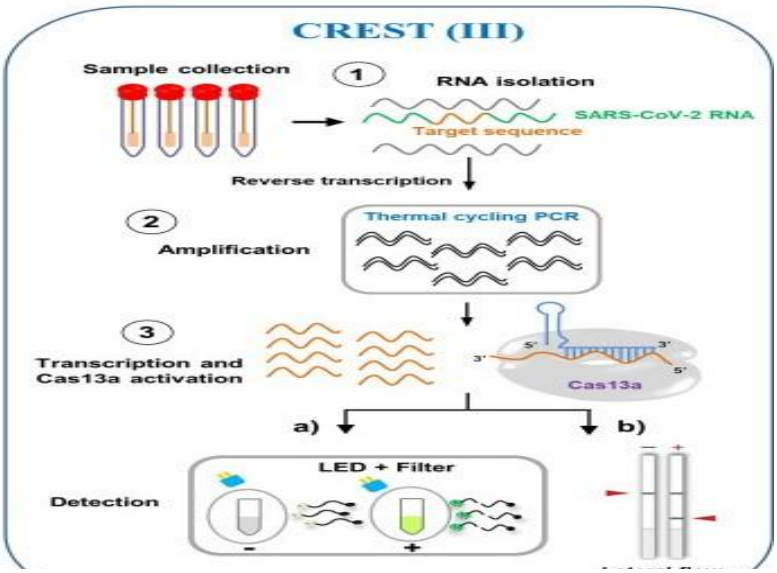
(Scheme 2). Single step SHERLOCK assays using extracted RNA with a fluorescent or colorimetric readout.

Building on this method, Arizti-Sanz *et al.*[22] coupled HUDSON and CRISPR -based programmability into one step to create SHINE (SHERLOCK and HUDSON Integration to Navigate Epidemics), a scalable diagnostics tool for detecting viral RNA from unextracted patient samples that has minimal equipment requirements and quick turnaround time. (Scheme 2). SHINE was validated on 50 nasopharyngeal clinical samples and shown to have 90% sensitivity and 100% specificity when compared to RT-PCR, and with a mean response time of 50 minutes. Employing SHINE SARS-CoV-2 in HUDSON-treated patient samples in nasopharyngeal swabs and saliva was detected with a paper base colorimetric readout or an in-tube fluorescent readout which can be performed with portable equipment and with reduced risk of sample contamination. The in-tube fluorescence readout and smartphone application used with SHINE allowed scalable, high throughput testing and automated interpretation of results. Thus, by reducing specialized personnel infrastructure, and time to obtain outcomes without sacrificing sensitivity or specificity.

- **CREST**

CREST- Cas13-based, rugged, equitable, scalable testing is another CRISPR based SARS-CoV-2 RNA detection method. This method was developed as an option for recurrent testing that could overcome issues with the supply of reagents and specialised equipment, and that is easy to deploy

at sites with minimal infrastructure. CREST addresses three of the main hurdles that limit the scalability of Cas13 based testing at sites with a minimal supply of resources: 1. Reagent accessibility, 2. Equipment availability and 3. Cost. By taking advantage of widely available reagents that are stable at room temperature, low-cost thermocyclers instead of isothermal reactions and easy to use fluorescent visualizers, this method bypasses the need for highly trained personnel and experienced molecular diagnostic laboratories.

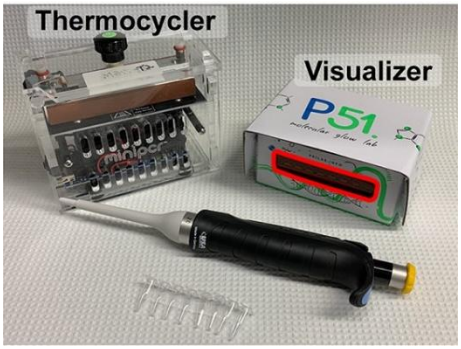


(Scheme 3). Workflow of CREST based detection of SARS-CoV-2

The CREST method employs standard sample collection, RNA extraction and reverse transcription. However, the amplification step uses cost effective Taq polymerases and portable thermocyclers. This has been made possible by creating inexpensive, Bluetooth-enabled thermocyclers and simple plastic filter-based LED visualizers. In the CREST platform, transcription and Cas13 activity are followed by fluorescence detection of de-quenched poly-U cleavage reporter, visualized with a blue LED (~495nm) and orange filter or other fluorescence detection system thus avoiding the use of expensive antibodies or antibody-conjugates for lateral flow immunochromatography. (Scheme 3)

Using CREST Rauch *et al*.[23] detected the presence of the N1, N2 and N3 sites in the SARS-CoV-2 nucleocapsid (N) gene, with an associated cost of reagents per unit test of US$ 5.90 compared to the PCR test cost of US$ 14 apiece. Thus, CREST is a relatively cheap format that allows portable diagnostic tests to access suspected cases without sophisticated instrument. The sensitivity of CREST was equivalent to the gold standard RT-qPCR and it can detect 10 copies of a target RNA molecule per microliter.

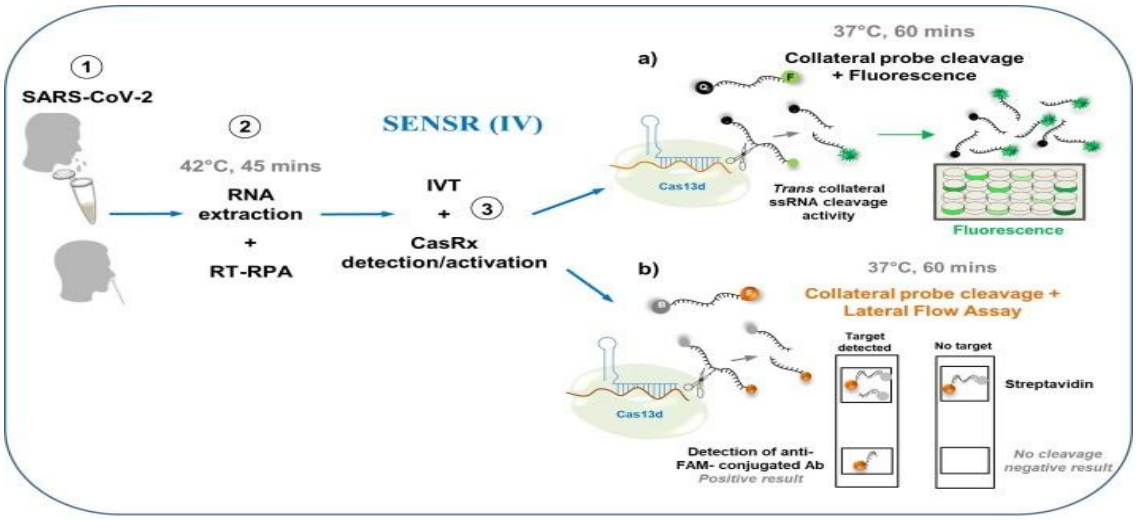
The RNA extraction step that is required for CREST, which uses commercial kits, is a bottleneck that might limit the accessibility and scalability of this method for detecting SARS-CoV-2 was overcome by coupling to a method called PEARL (Precipitation Enhanced Analyte Retrieval), a fast and accessible approach for the isolation of RNA, DNA and proteins.



(A)- the miniPCR mini16 thermocycler and P51 molecular fluorescence visualizer used, both of which are portable, can be operated with batteries and have minimal footprint. **(B)**- Fluorescent visualization of N1, N2, and N3 synthetic targets using P51 visualizer[23]

- **SENSR**

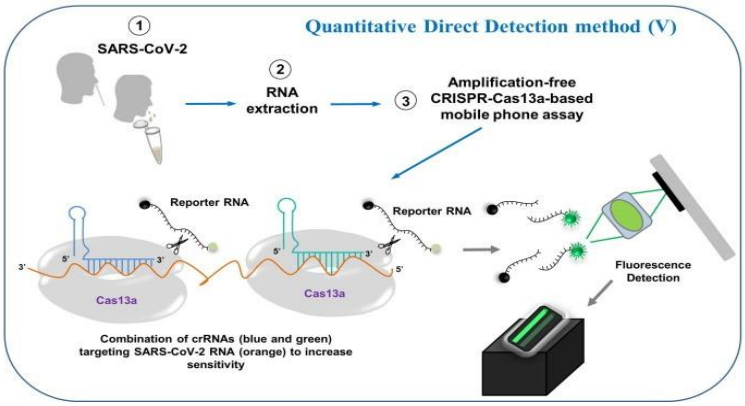
SENSR is a two-step protocol that requires an initial isothermal amplification reaction for 45 minutes combined with RT-RPA to produce short double stranded DNA amplicon containing a T7 promoter sequence. This CRISPR based nucleic acid molecular diagnostic tool harness the off-target collateral cleavage property of Cas13d ribonuclease derived from *Ruminococcus flavefaciens* (CasRx) to detect highly conserved SARS-CoV-2 viral sequences, in both synthetic templates and infected patient isolates. Results are obtained via fluorescence-based readout or lateral flow assay, and the total reaction time is just two hours. (Scheme 4).



(Scheme 4). Workflow of SENSR based detection of SARS-CoV-2

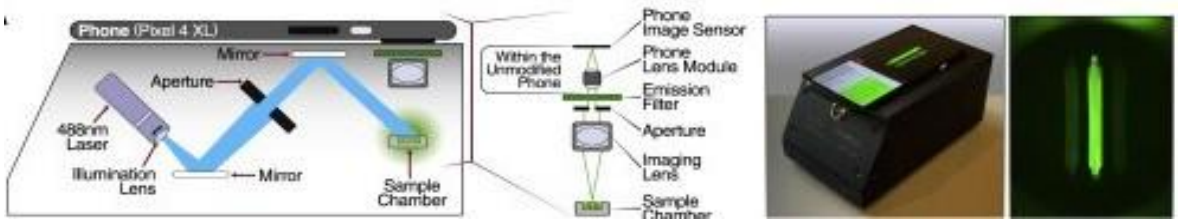
To determine the capability of SENSR to detect SARS-CoV-2 in infected patient samples, Brogan *et al.* performed fluorescence detection analysis on 42 RT-qPCR validated positive (n=21) and negative (n=21) patient isolates. SENSR yielded no false positive among the negative patient samples, demonstrating 100% specificity (0/21). In RT-qPCR analysis, a lower Ct value suggests a higher viral load within a patient sample. In this regard, SENSR detected SARS-CoV-2 RNA up to a maximum Ct value of ≤ 28, with a false negative rate of 25%. To examine whether SENSR can function as a point-of-care diagnostic test lateral flow analysis on 12 positive samples was performed, observing 92% concordance with SENSR fluorescence analysis. The LOD for SENSR was determined by fluorescence readout to be ~100 copies/µl.

Doudna *et al.*[24] developed a method for the direction of SARS-CoV-2 RNA that employs CRISPR-Cas13a without preamplification step for the viral genome. Unlike previous CRISPR-Cas13 diagnostics, this method is based on LbuCas13 homolog from *leptotrichia buccalis*, yields quantitative RNA measurements rather than only a positive and negative result. (Scheme 5). The detection of viral RNA takes place directly in the sample without additional manipulations. The strategy to increase the activity of Cas13a was through the use of multiple crRNAs along the N gene of SARS-CoV-2, which work in tandem to increase the sensitivity of the test. The rationale was that a single target RNA could activate multiple Cas13a ribonucleoproteins (RNPs). If each RNP is directed to different regions of the same viral target RNA, this could effectively double the active enzyme concentration. Thus, by taking advantage of crRNA combinations, two crRNAs were pooled in the same reaction, keeping the total concentration of Cas13a RNPs constant but divided equally between RNPs made with each crRNA. When both crRNAs were combined, both target detection and reaction sensitivity were increased markedly when measured with a fixed in vitro transcription (IVT) target RNA concentration (480 fM). This method allowed detection of ~ 100 copies/µl of SARS-CoV-2 viral RNA within 30 minutes, measuring changes in fluorescence over time rather than detecting only endpoint fluorescence.



(Scheme 5)

In recent years, smartphones have been adapted as diagnostic challenges that can replace some of the functionality of conventional laboratory equipment, simplifying diagnostic workflow of automating readout. In this regard the simplicity and portability of this method was demonstrated using mobile phone-based fluorescence microscope. (fig. d) This microscope measures the fluorescent signal generated by the Cas13a direct detection through a compact device, which includes low-cost laser illumination and collection optics. The functionality of this method was validated with SARS-CoV-2 positive samples extracted from nasal swabs. The accuracy was perfect, with positive results being detected directly with Cas13a was proportional to the concentration of target RNA in the sample, hence this method can provide speedy results quickly for patients with high viral loads.



(fig. d). Mobile phone-based microscope for fluorescence detection showing illumination and image collection components**(left).** Assembled device used for data collection and sample image taken by the mobile phone camera after running a Cas13a assay **(right).** [24]

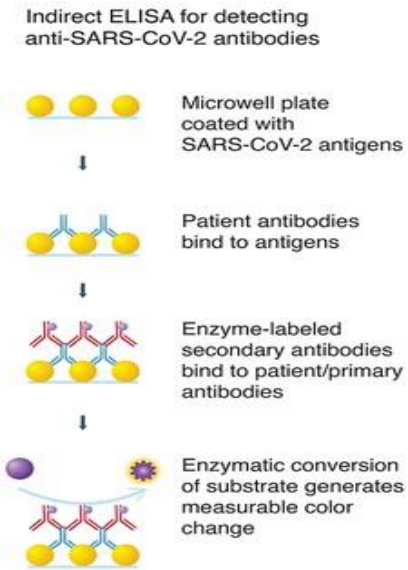
7. Detection of COVID-19 based on serological and immunoassays

Serological testing is defined as an analysis of blood serum or plasma and has been operationally expanded to include testing for saliva, sputum and other biological fluids for the presence of immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies. IgM first becomes detectable in serum after a few days and last a couple of weeks upon infection and is followed by switch to IgG. Thus, IgM can be an indicator of an early-stage infection and IgG can be an indicator of current or prior infection. IgM may also be used to suggest the presence of post-infection immunity.[4] 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. The methodology for these determinations includes the

traditional enzyme linked immunosorbent assay (ELISA), immunochromatographic lateral flow assay, neutralisation bioassay and specific chemosensors.

- **Enzyme linked immunosorbent assay (ELISA)**

ELISA is a microwell, plate -based assay technique designed for detecting and quantifying substances such as peptides, proteins, antibodies and hormones. The test can be qualitative or quantitative and the time to results is typically 1-5 hours. In the case of SARS-CoV-2, the plates wells are coated with a viral protein. If present, antiviral antibodies 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. (Fig a).[4]



(Fig a). ELISA assay detecting antibodies[4]

Zhang *et al*[25] detected immunoglobulin G and M from human serum of COVID-19 patients using an enzyme-linked immunosorbent assay (ELISA). They used the SARS-CoV-2 Rp3 nucleocapsid protein, which has 90% amino acid sequence homology to other SARS-related viruses. The recombinant proteins adsorb onto the surface of 96-well plates, and the excess protein is washed away. Diluted human serum is added for 1 hour, after which the plate is washed again. Antihuman IgG functionalised with horseradish peroxidase is added and allowed to bind to the target. The plate is washed, followed by the addition of the substrate 3,3',5,5'-tetramethylbenzidine. The peroxidase reacts with the substrate to cause a colour change that can be detected by a plate

reader. If anti-SARS-CoV-2 IgG is present, it sandwiches between the adsorbed nucleoprotein and the antihuman IgG probe, resulting in a positive signal.

ELISA is an inexpensive method, can be simply operated and made high throughput with an automated work station. However, this technique is time consuming and at a risk of contamination. It also needs infrastructure and qualified personnel.

- **Chemiluminescent Immunoassays (CLIAs)**

When an electron transits from an excited state to the ground state, visible or near-visible radiation is emitted, which is called luminescence. Chemiluminescence is a light-emitting process in which chemical reactions are the energy source for generating the electronically excited state. Immunoassays technique in which the 'indicator' of the analytic reaction is a luminescent molecule called chemiluminescent immunoassay.[26] This laboratory technique benefits from the specificity of the immune response and also the sensitivity of the luminescent reaction. It can be automated and quickly used on immunochemical analysers for a high throughput of samples. The principle of CLIA is the same as an ELISA with shorter incubation steps and no need for a reagent to stop the enzymatic reaction. In the first step of the chemiluminescence-based method, SARS-CoV-2 specific IgA, IgM or IgG in sera captured via SARS-CoV-2 antigen- N or RBD coated magnetic particles. Then, a second acridinium-conjugated antibody that detects human IgA, IgM or IgG was applied. Reaction of acridinium with its substrate caused strong chemiluminescence. Afterward, the diagnosed chemiluminescent signal over the background was measured as relative light units (RLU).

Examples of CLIAs

- **LIAISON SARS-CoV-2 S1/S2 IgG (Diarson, Saluggia, Italy)**[27]

LIAISON SARS-CoV-2 S1/S2 IgG assay, a standardized automated chemiluminescent assay, measured antibodies against the SARS-C0V-2 native S1/S2 proteins. The automated LIAISON SARS-CoV-2 S1/S2 IgG assay was examined in >1500 samples and its performance were sensitive, specific and precise with a high throughput capacity. The first results by automated LIAISON SARS-CoV-2 IgG assay are accessible within 35 minutes with a high throughput of 170 tests/hour. The sensitivity of the LIAISON SARS-CoV-2 S1/S2 IgG assay was significantly enhanced as the immune response matures.

- **Atellica IM SARS-CoV-2 total (COV2T) (siemens Healthcare, Erlangen, Germany)**

The COV2T assay is a chemiluminescent microparticle immunoassay (CMIA), which quantitatively measures total antibodies including IgG and IgM targeted to RBD SARS-CoV-2 in human serum and plasma. The results are obtained in 10 minutes on the Atellica IM Analyser with a capacity to process up to 440 assays per hour.

- **iFlash1800 (YHLO Biotechnology Company, Ltd, Shenzhen, China)**

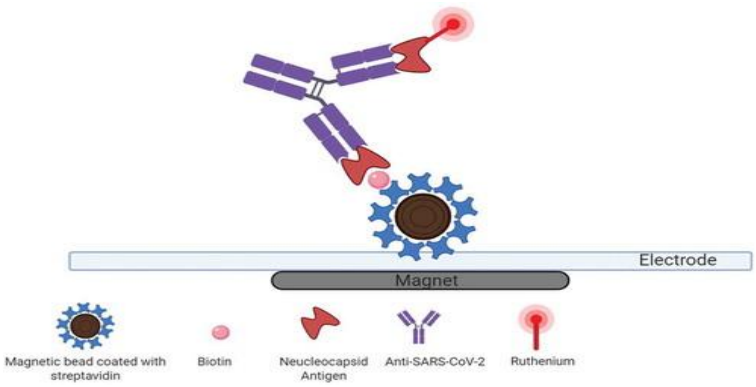
iFlash-SARS-CoV-2 is a paramagnetic particle CLIA for the detection of IgM and IgG antibodies against SARS-CoV-2 N and S proteins. The sensitivity of iFlash-SARS-CoV-2 was reported to be less than 50% before day 8 post-symptom onset, and it was increased to 81.8% 9-10 days after symptom onset for both IgM and IgG. A sensitivity of 100% was obtained 15 days or later after symptom onset when IgG was measured.

- **MAGLUMI (SNIBE- Shenzhen New industries biomedical engineering company, Ltd, Shenzhen, China)**[28]

The MAGLUMI 2019-nCoV IgG and IgM, indirect CLIAs on the fully automated MAGLUMI analysers, determine IgG and IgM antibodies against SAES-CoV-2 S and N proteins in human sera.

- **Electrochemiluminescence- based anti-SARS-CoV-2 immunoassay**

In the first step, the patient sample is mixed with biotinylated SARS-CoV-2 specific recombinant nucleocapsid (N) antigen and SARS-C0V-2 specific recombinant N antigen labelled with a ruthenium complex and then double-antigen sandwich immune complexes are formed. Next, streptavidin-coated microparticles are added and the complex binds to the solid phase via interaction of biotin and streptavidin. After transferring the mixture to the measuring cell, the microparticles are magnetically captured onto the surface of the electrode. In the next step, electrochemiluminescence is induced by applying a voltage to the electrode and then is measured by a photomultiplier. (fig. b).



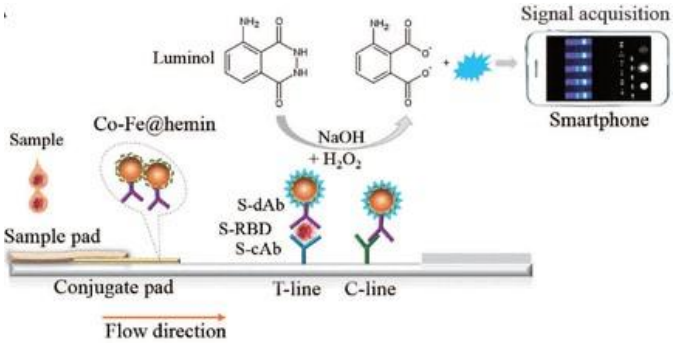
(fig. b)

- **Synthetic peptide-based Magnetic Chemiluminescence Enzyme Immunoassay (MCLIA)**

In the study of Cai *et al*. [29] twenty peptides from the orf1a/b, S, and N proteins were synthesized as candidate antigens and were labelled with biotin. Then, the biotinylated peptides were purified and bound to streptavidin-coated magnetic beads. Afterwards, serum samples were mixed with peptide-containing beads. Finally, after reaction with antibody conjugate and substrate, reactivity was determined by a chemiluminescent reader. Overall, a MCLIA based on the synthetic peptide from the S protein was developed for determining IgG and IgM against SARS-C0V-2. In the study conducted on sera of 276 patients with confirmed SARS-C0V-2 infection, the positive rate of IgG and IgM was 71% and 57.2%, respectively.

- **Nanozyme-based chemiluminescence**

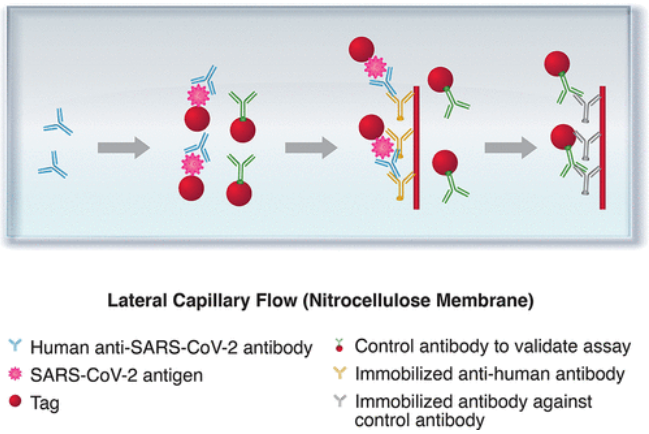
Liu *et al*. [30] developed a nanozyme-based chemiluminescence paper test for rapid, portable and ultrasensitive detection of SARS-CoV-2 RBD antigen by combining the high sensitivity of chemiluminescence, the high specificity of immunoassay and short testing time of lateral flow chromatography technique. This paper test is based on the principle of a double antibody sandwich immunoassay. First CoFe@hemin NPs labelled with S-dAb as the chemiluminescence probes were dispersed onto the conjugate pad. Along with the lateral flow of the sample, nanozyme probes combined with S-RBD antigen and cAb, forming the sandwich immunocomplexes. The nanozyme probes possess high peroxidases activity and thus, catalyse luminol substrate in the presence of H_2O_2 under alkaline condition, resulting in chemiluminescence, this chemiluminescence signal is then captured and analysed by a smartphone camera or CCD imaging system. (fig. c). The chemiluminescent intensity ratio of T- line to C-line is positively correlated with the concentration of S-RBD antigen. The signal amplification by nanozyme-catalysed reaction resulted in a great improvement in the sensitivity of paper test comparing to traditional colloidal gold, fluorescence strip or nanozyme colorimetric strip, comparable to that of ELISA.



(fig. c). Nanozyme-based chemiluminescence paper test for SARS--CoV-2 S-RBD antigen[30]

- **Lateral flow immunoassays (LFIAs)**

  This test is typically a qualitative (positive or negative) chromatographic assay. Samples move via capillary flow on the nitrocellulose membrane. When anti-SARS-CoV-2 antibodies are present, they bind to the labeled antigen and continue to move until they are captured by the immobilized antihuman antibodies. The presence of the captured antibody-antigen complex is visualised as a coloured test band. The labeled control antibodies comigrate until they are captured at the control band. (fig. d). The result of LFIA-based tests are displayed within 5-30 minutes.



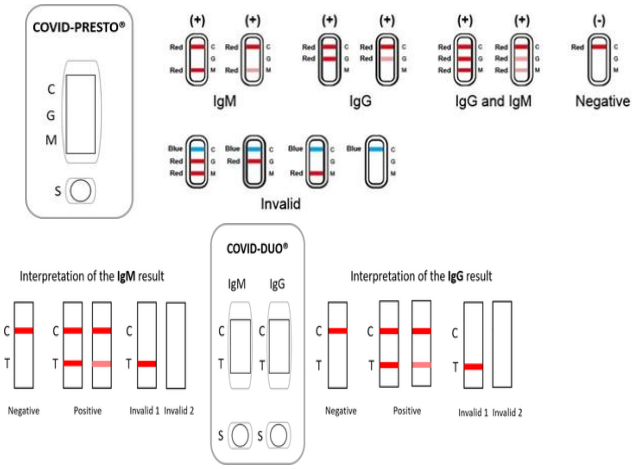
(fig. d). Lateral flow immunoassay for detection of anti-SARS-CoV-2 antibodies[4]

Example of LFIAs

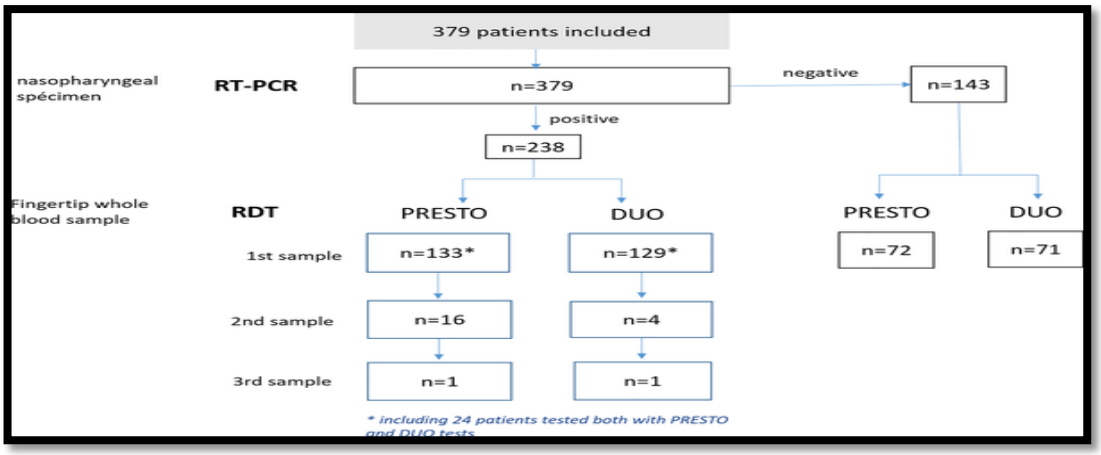
- **COVID-PRESTO® and COVID-DUO (AAz-LMB, Boulogne- Billancourt, France)**[31]

COVID-PRESTO and COVID-DUO are two SARS-CoV-2- IgG/IgM antibody test kits detecting N-protein targeted IgG and IgM antibodies in fingertip whole blood samples. (fig. e). Both are lateral flow immunochromatographic lateral flow assays. Anti-human IgM and IgG antibodies (test lines IgM and IgG) and rabbit IgG (control line) were immobilized on a nitrocellulose strip. Recombinant COVID-19 antigens labeled with colloidal gold were also incorporated into the strip. In 238 RT-PCR

positive COVID-19 patients, the specificity of both COVID-PRESTO and DUO RDT's were 100%, none false positive. The sensitivity of both of these RDT's increases over time from the onset of symptoms. The sensitivity of both RDT's reached 100% after 15 days from the onset of symptom.



(fig.e). Interpretation of results for COVID-PRESTO® (left) and COVID-DUO (right))[31]



(fig. f). Number of samples screened with RT-PCR and point-of-care tests

Simple operation, low cost and high speed are the advantages of LFIAs. However, the drawbacks of LFIAs are their low specificity and sensitivity.

CONCLUSION

In this literature review, we saw that Coronavirus can be classified into 4 genera: α, β, γ, δ and SARS-CoV-2 belongs to the genus β coronavirus which is comprised of crown-like, enveloped, positive single stranded RNA viruses. A total of 7 identification methods were reviewed out of which the most widely used was reverse transcription polymerase chain reaction (RT-PCR). But it

suffers from several limitations. Isothermal amplification method is a promising low-cost alternative and can complement or even replace PCR testing. MALDI-TOF method provides rapid detection with less than 1 minute. Detection based on biosensors and CRISPR-Cas method offers simple, rapid, reliable detection without the need for laboratory infrastructure. Moreover, the CRISPR-Cas method based on LbuCas13 homolog from *leptotrichia buccalis* provides both qualitative and quantitative detection**.** Apart from these molecular tests the serological and immunological tests provide valuable information about the course and degree of immune response. An accurate and immediate diagnosis of SARS-CoV-2 might be one of the most important lines of attack for controlling the spread of infection since it allows for the prompt isolation of people infected by the virus.

ACKNOWLEDGEMENT

I would like to express a deep sense of thanks and gratitude to my project guide Dr. Rupesh E. Patre sir for guiding me immensely and giving me a great opportunity to excel in my learning through the course of this project. I have achieved a good amount of knowledge through the research.

His constructive advice and constant motivation have been responsible for the successful of this project.

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