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Novel Coronavirus (COVID-19) diagnostics Tools: Current and developing techniques for detection

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Abstract
SARS-CoV-2 (2019-nCoV) is a class of novel type of corona virus which is responsible for the current COVID-19 pandemic. The scientists at global front are strategically taking steps to mobilize it and to reduce the rate of spreading infection. The major steps which are involved in curbing the spread of infections are early detection and contract tracing. Currently there are two major methods for detection of corona virus which is nucleic acid based testing and serological testing. Contact tracing involves series of steps involved in the identification of people who were in contact with the infected victim & providing them with information for isolation and preventing disease from spreading further. The prestigious World Health Organization (WHO) has only recommended the qRT-PCR based diagnosis as the golden standard for the detection of corona virus. In the current review we have briefly described about the existing methods for the detection of COVID-19 disease and also a wide knowledge on the emerging techniques in this field which has been used for the detection of similar diseases.

Keywords: SARS-CoV-2, COVID 19, Diagnosis, Pandemic & contact tracing

1. Introduction
The current outbreak of SARS-CoV-2 global pandemic which has led to a total of 3,621,245 cases of COVID 19 and 64,617 deaths in India, till to the date of 31 August, 2020 as per World Health organization reports [1]. It was declared as a pandemic by WHO on March 11, 2020 & all the organization have been immensely trying to reduce the spread of infection by their own precautionary measures. It was named SARS-CoV2 by the International Committee on Taxonomy of Virus on 11 February 2020. It is the seventh member of coronavirus family that is known to infect humans and other mammals [2]. It is the novel coronavirus recognized after the previous outbreak of SARS-CoV, MERS-CoV virus in year 2003 and 2012 respectively and causes fatal respiratory tract infections and pulmonary failure. Coronaviruses belongs to Coronaviridae family. It is a family of enveloped and single stranded RNA viruses which is further divided into 4 genera: alpha, beta, gamma, and delta. Current, SARS-CoV2 is a beta-coronavirus containing 29891 nucleotides and diameter ranging from 65-127 nm. It has 4 structural proteins including: S (Spike glycoprotein protein), E (Envelope glycoprotein), M (Membrane glycoprotein) and N (Nucleocapsid protein) [3]. It also contains some accessory proteins which are required for its replication. Since, the first case of COVID-19 was associated with the Huanan Seafood wholesale market, animal to human transmission is presumed to be the main mechanism of the first case reported for COVID-19 and various studies have surmised bats to be the key reservoir of this virus as SARS-CoV2 shares 96.2% identical genome with bats CoV RaTG13 [4]. Based on the study of these cases in Wuhan, CDC has proposed the incubation time for SARS-CoV2 within 3-7 days and the disease can last up to 2 weeks. The Basic reproduction number or R-naught value for this novel SARS-CoV2 virus is 2.2, hence each infected person can transmit this infection to 2.2 individuals further [3].
COVID-19 infected patients show various clinical symptoms like fever, dry cough, fatigue as well as shortness of breath. Serious complications are also seen in some patients including cytokine storm and acute respiratory distress syndrome which may cause death[5]. Recent study has also provided us with significant knowledge on the stability and survivability of the virus in aerosols (<5µm). It can survive for a maximum duration of 3 hours in air medium and on surface of plastic and stainless steel for up to 72 hours [6]. Hence due to these reasons the open environment and air acts as a common source for viral transmission. Hence early and accurate diagnosis of SARS-CoV2 is urgently required to confirm suspected cases, screen patients, and conduct monitoring of virus. Hence, we have summarized SARS-CoV2 testing methods in this review. These are divided into two parts: nucleic acid based and serology based methods. We have further categorized these testing methods into traditional and emerging techniques where we have discussed various traditional testing techniques that are already being used for SARS-CoV2 testing and the emerging techniques which have previously been used for the detection of related viruses and can be further validated for the detection of SARS-CoV2 as well.

2. Viral RNA based detection & diagnosis
The primary test which involves the detection of SARS-CoV-2 is carried out by Nucleic acid based diagnostic method. In this method the process of isolation of the nucleic acid from the host organism has been exploited and rapid amplification under controlled environment for the detection is being carried out. In January, World health organization (WHO) played an immense role in designing and distribution of first quantitative reverse transcriptase PCR tests (qRT-PCR). The entire genome size of SARS-CoV-2 varies from the size of 29.8 kb to 29.9 kb nucleotides in length. At the 5'end of the sequence more than the two third of the entire genome of it comprises of the orf1ab encoding or f1abpolyproteins, while at the 3' end one third of the entire sequence comprises of

genes which are responsible for coding of the structural proteins including the envelope, membrane, nucleocapsid and the surface of the SARS-CoV-2. In addition to these proteins it also contains 6 accessory proteins which are encoded by the genes ORF6, ORF7a, ORF7b, ORF3a and ORF8 8. This immense knowledge has been exploited by the researchers in for designing of primers and probes for the specific detection of this viral strain [7–9]. The standard procedure involved in the detection of the samples are as follows, 1) Sample collection from the infected host either by nasopharyngeal swabs (106-9 RNA/swab) or oropharyngeal swab (104-8 RNA/swab) [10]. 2) Sample transportation to the testing laboratory in the desired media such as Bovine serum albumin. 3) Isolation and purification of viral RNA by manual or kit based methods. 4) Amplification of Viral RNA by PCR for efficient detection. There is a general pattern of time consumed for performing these various steps. There has been search for an alternative PCR-based testing strategies because of the complexity and expenses involved in the currently available processes [11].

3. Current techniques

3.1 Real time Reverse Transcription-Polymerase Chain Reaction (RT-qPCR)

This technique is considered as the gold standard for the confirmation of SARS-CoV-2 virus in the upper respiratory samples because of its rapid detection, established usage, high sensitivity and specificity. The amplification and analysis of RT-PCR is carried out in a closed system, which provides it additional advantage and decreases chances of false positive results [5]. In RT-PCR, results are interpreted by Ct values. Ct value is the number of cycles required by the fluorescent signal to cross its threshold and become visible. In case of SARS-CoV2, Ct value of less than 40 is considered to be the indication of positive sample [12].

RT-PCR involves the conversion of RNA to cDNA by reverse transcriptase enzyme, followed by the amplification of specific regions of the cDNA by specific set of primers that can be monitored in real time using fluorescent dyes. RT-PCR can be executed as either one-step or a two-step assay. In a one-step assay, reverse transcription and PCR amplification are combined into a single reaction. On the other hand, in two-step real-time RT-PCR these reactions are performed in different steps. Although the two step approach offers the advantage of being more sensitive and flexible but one step RT-PCR is more suitable in this pandemic situation as it requires less time and labour [12–14].

Enormous studies are being carried out by the researchers worldwide to optimize the protocol for RT-PCR. Corman *et al* and his team has optimized the protocol for real time RT-PCR for the identification of SARS-CoV2 in clinical samples as well as cell culture supernatants. RNA was extracted from clinical samples using MagNA Pure 96 system (Roche, Penzberg, Germany) and from cell culture supernatant using viral RNA mini kit (QIAGEN, Hilden, Germany). One step RT-PCR was done using platinum Taq polymerase (Invitrogen, Darmstadt, Germany) and E gene was described as the first line screening tool followed by RdRp gene [15]. Xiao *et al* and his team has used real time RT-PCR to detect SARS-CoV2 in Throat or deep-nasal-cavity swab samples over different time period. Two target genes Orf1ab and N were tested using COVID-19 detection kit manufactured by (Shanghai Huirui Biotechnology Co, Ltd). It was observed that positive rate of RT-PCR result was highest at week 1 (100%), followed by 89.3%, 66.1%, 32.1%, 5.4%, and 0% at weeks 2, 3, 4, 5, and 6, respectively [16].

In addition to this, Wenling Wang, and his team has collected different samples (pharyngeal swab, blood, sputum, urine, nasal swab, bronchoalveolar lavage fluid and fibrobhronchoscope brush swab) from COVID-19 positive patients to check for positive rates. In this study, it was observed that bronchoalveolar lavage fluid showed the highest positive rate of 93% followed by sputum (72%), nasal swabs (63%), fibrobhronchoscope brush swab (46%) pharyngeal swab (32%), faeces (29%), blood (1%), urine (0%) [17].

Despite being the most widely used method for detecting SARS-CoV-2 infections, it has certain disadvantages as it requires expensive laboratory reagents, instrumentation and chances of false negatives results due to sampling errors. To reduce this instrumentation requirement and time, a modified rapid RT-PCR has been designed using thin-walled PCR tubes and a setup including sous vide immersion heaters /circulators which can perform 40 cycles of RT PCR in just 12 minutes [14]. The disadvantage of this approach is that a non-multiplexed reaction algorithm uses larger volumes of reagents. Also, the reaction will take up to 30 minutes with regular PCR tubes [18].Another modified version of qRT-PCR is based on direct fluorescent PCR capillary-electrophoresis detection of the viral genome. Here, primers are labelled with fluorochrome at 5´-end. The products of PCR reaction are subjected to capillary electrophoresis in ABI3130 sequencers to visualize the fluorescent peaks. This method takes less than 5 hours to test 96 samples [19]. Despite of being the gold standard method, further advancement is required to improve RT-PCR to successfully employ it. The overall process has been well explained in figure 1.

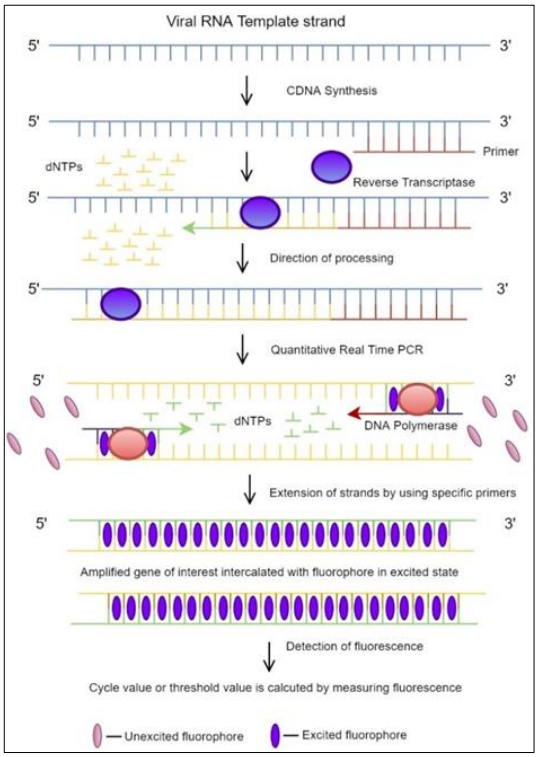

Fig 1: The diagram represents the overall process for the detection of the desired gene by using the complex process of RT-qPCR. The major steps involved in the process is synthesis of cDNA and then the next process is qPCR which shows us the presence or absence of the desired gene of interest.

3.2 Isothermal nucleic acid amplification

Loop-mediated isothermal amplification (LAMP) is a technique developed in recent years which exploits DNA as a substrate for amplification and helps in identification of gene of interest in the sample pool. This technique was developed in contrast to the conventional PCR technology. In this entire process is carried out at a single constant temperature without a need for thermocycler. This process is efficiently combined with Reverse transcription reaction for the detection of desired RNA [20].

To curb the drawback associated with the use of RT-PCR, Notomi *et al*, developed a novel method of DNA amplification named loop mediated isothermal amplification in the year 2000 [20]. This method mitigated the need of traditional thermo cyclers to maintain temperature changes and allows the rapid, efficient and specific amplification of DNA at the constant temperature. This method can be carried out in a single tube using 6 highly selective internal primers for 6 different target sequences simultaneously. All the steps of this reaction from multiplication to detection can be carried out in a single tube at a constant temperature. This technique can directly utilize RNA or combined with Reverse transcriptase reaction to covert the RNA into cDNA and then its detection[21]. The amplification product can be detected via photometry by measuring the turbidity caused by magnesium pyrophosphate precipitate in solution as a by-product of amplification. The reaction can also be followed in real time by measuring the turbidity or by fluorescence using intercalating dyes.

Moreover, LAMP diagnostic testing requires only heating and visual inspection, its simplicity and sensitivity makes it a promising candidate for virus detection [22–24]. Numerous studies have now shown the successful application of LAMP assays in various forms to detect SARS-CoV-2 RNA in patients' Samples. Ganguli *et al* and his team has reported a portable, rapid and POC method of diagnosis of SARS-CoV2 from clinical samples. This method relies on the use of cartridge and smartphone, the real time monitoring of fluorescence emission during amplification is done using smartphone camera. This method is capable of giving results in 30 minutes with limit of detection of 50 RNA copies per microliter in viral transport medium solution [25]. Lin Yu and his team has optimized RT-LAMP assay protocols for the rapid detection of SARS-CoV2 virus in clinical samples. The primers were designed for ORF1ab gene as a target gene for the detection of SARS-COV2. This method was capable of detecting as low as 10 copies of ORF1ab gene [26]. Yan et. Al. has used 130 suspected SARS CoV-2 patients and tested the performance of this assay with other 60 respiratory pathogens including all other human coronaviruses. They observed 100% sensitivity and specificity with LOD of 10 copies per µl [23, 27].

Despite being a simple sensitive assay for detection, it holds many disadvantages also which are: Complexity associated with the design of multiple primers, selection of suitable regions in the gene for primer designing, formation of complicated products with different sizes. Chances of unexpected signals derived from primer dimers or non-primer reactions. Since, this technique is not conventional as RT-PCR, more validation for this technique is required [28].

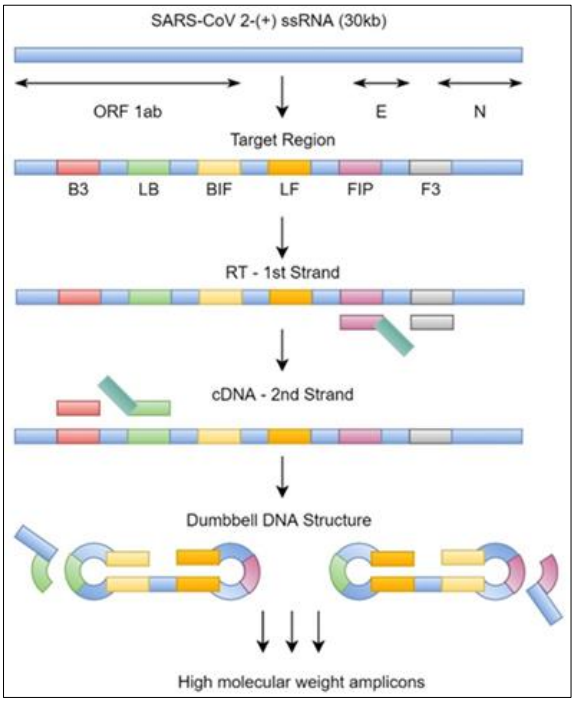

Fig 2: The basic work flow of Isothermal nucleic acid amplification is given in the above diagram. RT-LAMP uses more than two set of primers namely: Forward Inner Primer (FIP), Backward Inner Primer (BIP), External primers B3 & F3, loop specific primers: Forward loop primer (LP), backward loop primer (LB). FIP in DNA pattern will attach to complementing region to pattern strand and will start the synthesis of complementary strand. External primers F3, B3 along with the help of DNA polymerase of Bacillus stearothermophilus then elongates the strand. This displacement cycle continues on the other end of the DNA also. As the strand synthesized by these primers contains two complementary regions FIP & F3. These can now bind to each other and cause the newly formed sequence to form dumbbell like structure with 2 loop structure. This dumbbell structure forms the basis for the cycling phase of the LAMP reaction. Binding of the FIP primer to the FIP complementary region and self-priming of the region results in further synthesis. This round of synthesis results in the release of self-priming single stranded structure with sequence complementary to that of the initial dumbbell and to which BIP primer can bind. This leads to the production of huge number of increasingly complex and variously sized stretches of high molecular weight amplicons. The image was made in reference to the research work of Linda et.al [14].

3.3 Amplicon & Metagenomics based Sequencing: Amplicon based sequencing is done to provide an insight immense knowledge on the viral genome at the basic base pair level information. In this method a set of specific primers are utilized for amplifying the required gene of interest [29]. It also provides us with the knowledge of molecular epidemiology, potential contact tracing and studies of viral evolution. Metagenomics is a technique which is used for explore and analysing the microbiome of an infected individual. When this duel techniques are combined together as a diagnostic tool in provides a rapid insight for the identification of major culprit SARS-CoV-2 and the other pathogenic partners which plays an essential role in the contribution of secondary infection. It also provides us detailed information on mutation and the ways it interacts with other human coronaviruses. These significant data will enable us for the development of antiviral vaccines at a much faster rate [14].

This idea of utilizing both the metagenomics & amplicon based sequencing (MinION) approaches has been utilized by Moore *et al*. (2020) for the rapid detection of SARS-CoV-2 genome and its corresponding other microbiome partners in nasopharyngeal swabs within a short interval of 8 hours. A major 16 primer binding sites were selected in the SARS-CoV-2 genome which acts as the conserved regions for amplicon based system. Then approximately around 1000bp fragments with a 200bp overlapping regions were amplified. Subsequently these primers are used for MinION method which uses shotgun metagenomics approach for analysing the background information for the presence of other pathogenic partners [14, 30].

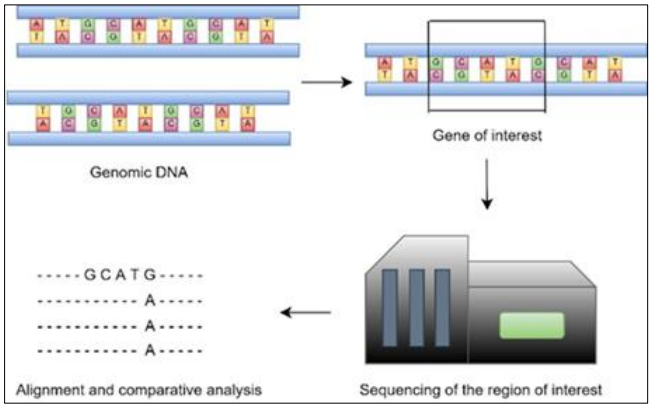

Fig 3: The workflow diagram of sequencing and identification of desired region of study has been highlighted.

4. Developing techniques

As the rate of COVID19 infection has been significantly expending on a regular basis and have reached a scale of 26 million cases as of September 2020, there has to be a more rapid, robust and cost efficient way for the detection[1]. Designing of a device which can be used to identify infection on the onsite region of infection or can be used for self-diagnosis by common citizens, will be a major breakthrough on the diagnosis field. This part of the review consists of the recently developing techniques which have been already employed for the detection of other respiratory viruses. If these techniques can be combined with the essential parameters required at the current time, these techniques can hold an up hand in future for COVID19 detection.

4.1 RT-RCA: Rolling circle amplification (RCA) is a unidirectional nucleic acid replication process which uses a nucleic acid template at isothermal conditions with the help of DNA or RNA polymerase for the rapid synthesis of multiple copies of circular molecules which can either be DNA or RNA. Few common examples of products amplified this method are plasmids, circular RNA genome of viroids and genome of bacteriophage[31]. The main mechanism involved in the process is that the nucleotides are added continuously to the primer which is annealed to the circular template by polymerase. This results in the formation of a long concatemer ssDNA that will contain tens to hundreds of tandem repeats which is complimentary to the circular template [32]. In addition, a large group of multifunctional materials with diverse properties can be used for hybridizing RCA products with complimentary oligonucleotides tethered to functional moieties. Some of the common used moieties are fluorescent dyes, antibodies, enzymes and nanoparticles which used for sensitive detection [32].

Seyed Vahid Hamidi et.al, has shown that this method can be used for Real-time detection of H5N1 influenza virus through hyper-branched rolling circle amplification with analytical sensitivity in the concentration range from 10 fM (femto Molar) to 0.25 pM (pico Molar) and detection limit of 9 fM. They have also compared the assay with standard methods available and reported that this method is more robust, rapid and has an ultra-high sensitivity[33]. Martel and his team have also showed the usage of RCA application in Hepatitis B virus. They have used the relaxed circular DNA of virus and performed in vitro amplification of full genome [34].Bin wang *et al* and his team has also employed RT-RCA for the detection of SARS-CoV in culture medium and clinical samples. This method was observed to show high sensitivity in both liquid and solid phase. One of the major drawbacks of the method is the formation of non-specifically amplified products; extremely optimized conditions are needed to reduce it [35].

4.2 RT-RPA: RT-RPA stands for Reverse Transcription-Recombinase Polymerase Amplification, is another single tube, isothermal nucleic acid amplification [36]. This technique employs three main enzymes which performs three steps of process. First, Recombinases which pairs up the oligonucleotide primers with homologous sequence in duplex DNA. SSB i.e. Single strand binding proteins that bind to displaced strands of DNA and prevent the primers from being displaced. Finally, the strand displacing polymerase which synthesizes the target DNA [37-38]. For detection of amplification product, it can be coupled with fluorescent probe or lateral flow strip detection [39]. It has been shown to detect much lesser viral load than RT-PCR or LAMP method and simpler to design than LAMP.

Amer et. Al. has shown the detection of Bovine Coronavirus using RT-RPA method with analytical sensitivity of 19 molecules in 10-20 mins. Sixteen faecal and 14 nasal swab specimens were collected from cattle showing intestinal and/or respiratory manifestations and used for RPA based detection. They have also compared the assay with standard RT-PCR and found to be more rapid. For point-of –care application, they have combined this assay with new portable fluorescence reader weighing only 1.2 kg [37]. However, as compared to LAMP which can tolerate inhibitors, RT-RPA is sensitive to contaminants and shows off target products leading to less specificity. Yuan Li *et al* and his team has also used RT-RPA for the detection of peste des petits ruminant's virus in clinical samples. Detection limit of 14.98 copies per

reaction was observed. As compared to RT-PCR, RT-RPA showed 100% sensitivity and 97.80% specificity [40]. Behrmann *et al* has validated the use of RT-RPA for SARS-CoV2 detection. Total viral RNA derived from cell culture supernatant and 19 nasopharyngeal swab samples were screened. This method has shown 100% sensitivity and is simple to use alternate to RT- qPCR [41].

4.3 DNA Barcode: In the year 2003, Dr. Hebert from the eminent University of Guelph, Ontario, Canada, devised a new emerging technique called DNA barcoding. This method involves series of process in which the short DNA sequence is recovered from a standardized and well characterized part of the genome. The obtained sequence of barcodes from each unknown specimen was then compared with the sequences available in the recognized library identity. It is one of the obligatory tool which is accurate and fast for species level identification by use of short DNA sequences [42]. There are two major strategies involved in designing of this method which is, the first one is Positional encoding. In this process every potential reaction is already pre-assigned a particular position on a solid-phase support such as a DNA microarray. The second strategy involves reaction encoding in which the every possible reaction was assigned a uniquely tagged code which can be either particle or optical based [43]. Yougen Li et.al, showed that fluorescent DNA Nano barcodes can be used for detection of multi-pathogen DNA. They have developed a system in which a small fragment of characteristic DNA from the species of B. anthracis, F. tularensis, Ebola virus and SARS coronavirus were selected for the study to be target DNA. The barcoded probes has different fluorescent intensity based on the target DNA detected, this fluorescence has been noted and compared with the coded library to obtained precise information on the infection. DNA barcoded were multiplexed with dot blotting, flow cytometry and fluorescence imaging which shows that the results of detection are highly sensitive, more rapid and reliable. Few drawbacks available in this method is optimization of micrometre size, biocompatibility & polydispersity plays a crucial role in fabrication process [43].

5. Serological detection of SARS-COV2

Detection of infectious virus using nucleic acid based diagnostic approaches is increasing, owing to this pandemic situation. Amongst all the nucleic acid based methods, RT-PCR is being used as the 'gold standard' for the detection of this highly contagious virus. Despite its high sensitivity and specificity, using RT-PCR as a sole diagnostic method for the confirmation of SARS-COV2 in a patient faces some limitations. RT-PCR can only detect acute infection when the virus is actively replicating inside and the presence of inhibitors of PCR which if not properly removed during extraction of the viral nucleic acid hinders the process of detection using RT-PCR yielding false negative results [44]. Since, SARS-COV2 virus has high mutation rate and mutations in the target gene sequence, improper sampling, can also yield false negative results [45]. Considering all these limitations, development of alternate methods of diagnosis like serological assays for the detection of such infectious virus becomes a need of an hour. Serological assays measures the antibodies generated by our body in response to any infection. Antibodies are glycoproteins which are generated by B cells in response to an immunogen. An immunogen guides the B cells to get converted into antibody secreting plasma cells. 5 types of antibodies are generated in humans IgM, IgG, IgA, IgD, IgE [46]. Most of the serological tests measures either IgM or IgG antibody and are based on capture method where target antigen/antibody is immobilized on the surface which due to their affinity binds to the respective analyte present in the test sample. These immunocomplexes thus formed, are later on visualized using different kinds of labels including enzyme, radioactive, fluorescent etc [47]. IgM being the first antibody produced when a person is exposed to an infection, reflects primary infection. IgM has low affinity and shows cross reactivity with related epitopes of various CoVs. Generation of IgM is followed by IgG which shows high specificity and remains in the circulation for longer period. Since, antibodies can be detected for a long time after an infection, detecting it, will assist in the epidemiological interrogation and helps to track the transmission chain and possible source of the infectious virus. It enables us to find people who have recovered from the past or recent infection, to identify and isolate them and their close contacts so as to stop the further spreading of virus [48].

In the following sections, we will briefly discuss various established as well as emerging serological methods for the detection of SARS-COV2, as well as advantages and disadvantages associated with their use.

5.1 ELISA: This is the most extensively used assay for the detection of various antigens, antibodies and proteins. It is a quantitative or semi-quantitative method which works on the basis of antigen-antibody interactions[49]. It is generally carried out in a 96 well microtiter plate which allows the simultaneous detection of multiple samples. It is further divided into 3 types: Indirect, Sandwich and competitive ELISA. ELISA involves the immobilization of an antibody/antigen onto the surface of a microtiter plate, followed by the addition of a test sample to detect target analyte. It involves an enzyme labelled antigen or antibody. Most commonly used enzymes in ELISA include horseradish peroxidase, alkaline phosphatase and beta-galactosidase etc. On the addition of the chromogenic substrate, colour development takes place. This colour development signifies the presence of antigen or antibodies in indirect and sandwich ELISA whereas absence of the same in competitive ELISA indicates the presence of the target [50].

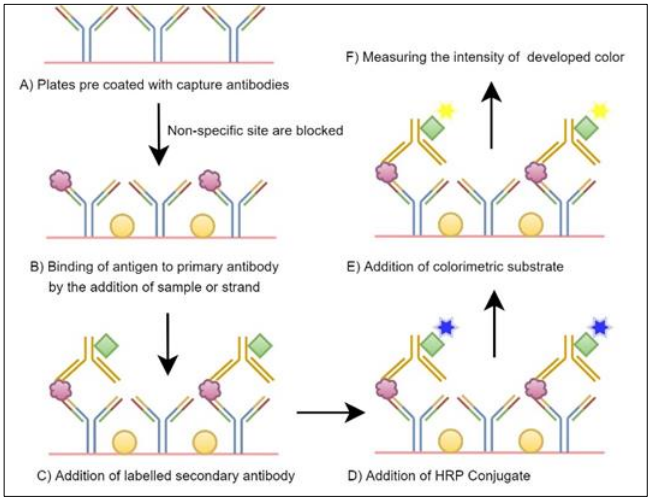

Fig 4: The figure depicts the major steps involved in the ELISA method. ELISA can be modified according to the user necessity. In the above mentioned diagram Antibodies are coated on the surface. Samples collected from the host is used as the binding antigen in the sandwich ELISA.

Zhao *et al* and his team has used ELISA kit manufactured by Beijing Wantai Biological Pharmacy Enterprise Co, Ltd to determine the antibody response in patients infected with SARS-COV2 virus. The detection specificity obtained using this kit for total Ab, IgM: IgG were 99.1%, 98.6%, 98.6% respectively. This kit offered variable sensitivity depending upon the day of onset of symptoms[51]. Lau K. P *et al*, and his team used also used ELISA for the detection of SARS-CoV2 nucleocapsid protein in nasopharyngeal aspirate, urine and faecal sample of infected patients within day 2-23 after the onset of illness. This assay showed high specificities of 96.7, 99 and 96% respectively. Researchers were able to detect nucleocapsid protein from day 6-24 in nasopharyngeal aspirate, 11-31 in urine sample and 8-32 in faecal samples [52]. Application of ELISA for the diagnosis of such infectious diseases offer high sensitivity, specificity, safe and eco-friendly platform requiring no radioactive reagents whereas, its transportation and storage at low temperature and cost of antibody production poses a limitation on its use [50].

5.2 Neutralization assay: Class of antibodies produced in our bodies in response to a viral infections is known as Neutralizing antibodies and they play an important role in virus clearance. Hence, evaluation of titers of neutralizing antibodies in patients who have recovered from SARS-COV2 virus is important before using their sera as a therapy for those who are still infected [53]. To perform neutralization assay, pseudoviruses are used and they serves as a major tool in virology to study emerging and re-emerging viruses. These are used to determine the titers of neutralizing antibodies present in the convalescent sera of a patient infected with any virus, eliminating the risk of handling infectious viruses by researchers and provides ease to accomplish studies for vaccine development in BSL-2 facilities [14]. These assays are usually carried out in using pseudovirus infected cell cultures where convalescent sera of a patient is serially diluted and is added at a decreasing concentration. Neutralizing antibodies if present, inhibits the replication of virus and their titers can be determined by the EC50 values. EC50 is the serum dilution at which relative light units are reduced by 50 percent as compared to the control (virus+cells) after subtracting background signals from cells only group [54]. Nie*et al* and his team has optimized the protocol for the neutralization assay of SARS-COV2 virus including cell type, viral inoculum, and cell number. This standardized protocol will help researchers worldwide towards designing various therapeutics or vaccine for this infectious virus. For optimizing the entire protocol, VSV pseudotyped system was used to create SARS-COV2 pseudovirus. S gene of SARS-COV2 virus was cloned into a eukaryotic expression vector to generate recombinant vector. VSV G pseudotyped virus provided the backbone and had expression cassette for luciferase gene. This pseudovirus were transfected in different cell lines were screened and Huh7 cells were identified as the best to study this virus infection [54]. Fan Wu *et al* and his team further evaluated the titers of antibodies in patients recovered from SARS-COV2 by infecting 293 T/ACE2 cells using pseudoviruses. They observed that the neutralizing antibody levels reaches their maxima after 10-15 days of the disease onset. The antibodies that targets the RBD, S1, S2 subunits of Spike proteins were the ones majorly responsible for neutralization of the virus [55].Tian *et al* and his team also reported a pseudoviral system with a bioluminescent reporter gene to detect the antibody titers in response to a vaccine for H7N9.In spite of all the advantages, major drawback of this assay is the requirement of technical staff and inability to use this for point of care diagnosis [56].

5.3 Lateral Flow Assays: This is a paper strip based innovation and is used for the qualitative and quantitative detection of a target analyte. Using LFA for detection of this infectious virus possesses various advantages as it is portable, cost-effective, requires no laboratory processing, uses low sample volume, easy storage This assay has the potential to provide point of care diagnosis and gives result in 5-30 minutes[57]. Because of the ease of interpretation of results using LFA, its usage has been expanded to various fields like agriculture, hospitals, and food industries to detect various toxins, pathogens, pollutants and chemicals. This paper strip is composed of overlapping membranes placed on an inert back card generally nitro-cellulose membrane which imparts stability to it. LFA are typically comprised of: sample pad, conjugate release pad, membrane affixed with antibodies and adsorbent pad. The patient sample is deposited at the sample pad which is generally impregnated with buffer salts, surfactants and proteins. The sample via capillary force flows to the conjugate pad which holds the detector particles which are antibodies conjugated with nanoparticles. These conjugated antibodies binds to the target analyte and migrates towards the test line where it binds the anti-analyte antibodies giving a visual result. A positive result is obtained when the analyte binds to the captured monoclonal antibodies in addition to the detector antibodies giving a visible color to the test line [58-59]. Xiang *et al*, and his team has evaluated the sensitivity and specificity of the combined Colloidal gold immune-chromatographic assay (GICA) IgM and GICA IgG detection kit manufactured by Zhu HaiLivZon Diagnostics for the detection of SARS-COV2 and is observed as 82.4%, 100% respectively [60].Similarly, Phan *et al* and his colleagues have evaluated the efficacy of LFA developed by Naval Medical Research Centre for the detection of glycoprotein sequence of Ebola virus disease. Plasma samples and post-mortem oral swabs of patients of Ebola virus were taken. The accuracy reported for plasma sample, oral swab and plasma and oral swab taken together were 95.9%, 95.8% and 95.8% respectively [61]. In advancement to this studies, Oem *et al* and his team has also designed a lateral flow assay based on monoclonal antibody (MAb70-17) for the detection of foot and mouth disease and had compared it with traditional AgELISA assay. The former offered sensitivity and specificity of 87.3% and 98.8%compared to 87.7% and 100% offer by AgELISA. LFA showed comparable results to the most widely used method for detection (ELISA) and is capable of providing immediate results[62].Thus, using LFA for disease diagnosis helps to implement viral control procedures swiftly in the area of viral outbreak. In-spite of all the advantages, there is further need of improvement in this assay to increase its sensitivity, use of new labelling and reading technologies, so as to provide more accurate and reliable diagnosis to a patient, mitigating the need of result confirmation using standard laboratory procedures [63].

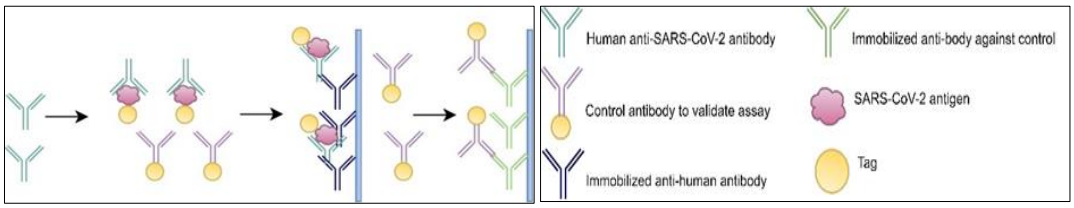

Fig 5: The figure represents the LFA for the detection of anti SARS-COV2 antibodies in patient's sample: This paper strip consists of overlapping membranes placed on inert back side. If anti SARS-COV2 antibodies are present in patient's sample, they flow to the labelled antigen via capillary forces and then they reach immobilized anti-human antibodies. This antibody-antigen complex is then visualized as colored test band. The labelled control antibodies co-migrate until they reach control band. The picture was made in reference to the research work of Linda et.al, [14].

6. Emerging Serological techniques

Due to the current lack of vaccine for this highly contagious virus, a foremost step towards controlling its spread involves the timely or early diagnosis. Though the use of traditional detection approaches provides fairly high sensitivity and specificity, but they are associated with certain limitations. These diagnostic assays requires transportation of the patient's sample from their respective places to the laboratories, absence of well-established laboratories in resource-poor areas makes it very time consuming. Further processing of patient's sample by expert technicians, retrieving reports and follow-up also requires additional days. Hence, there is an urgent need for the novel diagnostic methods which can speed up the detection process to firmly strategize disease control, can provide point of care diagnosis to a patient and are cost effective for the general population. This could be achieved either by modifying existing diagnostic methods like RT-PCR, ELISA, re-using the functions of known devices (smartphones), conjugating various traditional assays etc[64]. Enlisted below are some of the emerging techniques in the field of diagnosis of infectious viruses which provides POC diagnosis, requires small volume of sample, have multiplexing ability.

6.1 Immunofluorescence assay

It is a robust microscopy technique employed in virology to identify the presence of antibodies in patient's sera for a particular viral infection, by its ability to react with the viral antigens expressed in the infected cell culture[65]. IF assay is further divided into two types depending upon the staining method employed: Direct and indirect immunofluorescence. Direct immunofluorescence follows relatively simple procedure, where fluorophore is directly attached to the primary antibody. On the other hand, indirect immunofluorescence assay is more sensitive, as it is a 2 step approach which uses labelled secondary antibody and provides greater amplification of the signal [66]. Inoue *et al* and his team has reported IF assay for the detection of herpesvirus-8 specific antibodies in human sera. Because of the absence of any gold standard primary effusion lymphoma (PEL) cell line was used as a reference. BHK-21 cells infected with semliki forest virus were used to express specific proteins of HHV-8 (ORF73 and K8.1). K8.1 IF yielded better results as compared to the ORF73 and showed sensitivity and specificity as 94 and 100% respectively [67]. Immunofluorescence was also used by Chan *et al* and his team for the detection of SARS. They infected vero cells with SARS-COV virus, harvested cells after 96-100 hours and then mixed it with non-infected vero cells at the ratio of 1:1. The slides and the serum samples were processed according to the protocols and then a fluorescein isothiocyanate – conjugate rabbit anti-human IgG antibody was added and incubated for 30 minutes at 37 degrees. This indirect fluorescence measurement of anti-SARS-COV virus in cells showed 100% sensitivity and specificity. However, the only drawback is the requirement of experienced technicians to carry out this assay [68].

6.2 Smart phone based assays

Smartphones are small gadgets that have revolutionized the way humans connect with one another and because of its growing use in developing areas, researchers are now interested in employing this existing technology for its use in the field of diagnosis of infectious diseases [69]. Laksanaopin*et al* and his team has come up with a lab- on-a-chip setup that made it possible to perform a laboratory quality ELISA on smartphone using whole blood of an individual obtained via finger pricking. They have used this approach to detect antibodies to 3 infectious viruses HIV, treponemal-specific antibody for syphilis and nontreponemal antibody for active syphilis in a single go. This POC device 'dongle' is a versatile, portable, easy to use and cost effective device into which microfluidic cassette is inserted. This device can be connected to 4th generation Apple i-pod touch and was capable of working with very low power consumption and giving result in 15 minutes. The audio jack of smartphone provides it enough audio signals that are converted into stable voltage, which eliminates the need of battery. A microfluidics test cassette was designed with multiple detection zones for different infectious viruses and it has all the reagents like lyophilized secondary gold conjugated antibodies placed in an antibody holder along with the wash buffers and silver ions pre-loaded on the cassette for signal amplification. This device works mechanically and has one push vacuum chamber built by using rubber bulb. On pressing rubber bulb, negative pressure was generated due to which the sample starts to flow through the cassette to the sequentially placed reagents on it. The dongle measures the OD values after the silver enhancement assay and data transmission was done by Frequency shift key which transmits the data as high or low frequency wave back to the smartphone via audio jack, these frequency waves are converted into numbers via app installed on the smartphones. The sensitivity and specificity offered by smartphone based diagnostic methods are 92-100 % and 79-100 % respectively[70]. Smartphone based diagnostics is of immense benefit in resource poor areas where laboratory setup is not good but at the same time inefficient power supply in these areas may pose difficulties in charging smartphones [71].

6.3 Single molecule ELISA

Several serum biomarkers have been exploited for the early

detection of diseases, in order to ameliorate the disease prognosis. However, low abundance of these protein secretion during the initial stages of a disease poses a limitation on its detection using conventional ELISA [72]. The use of traditional ELISA for the detection of such protein biomarkers could yield false negative results as the fluorescence of single enzyme gets masked in large assay volumes. Hence, there is a need for the refinement of such assays to increase their detection sensitivity [73]. Rissin*et al* and his colleagues have reported a new approach called 'digital ELISA' for the detection of single protein molecule at sub femtomolar concentrations using femtoliter sized reaction chambers which they termed as single molecule array (SiMoAs). They have used this method for the determination of PSA antigen in patients who have undergone radical prostatectomy and it offered extremely high sensitivity (14 fg/ml) by magnifying signal/noise ratios. This assay involved the addition of sample onto the paramagnetic beads coated with antibodies on their surface which were subsequently labelled with secondary antibodies to form sandwich of antigen/antibody, large number of beads used in this assay ensures the binding of all the target protein molecules as single protein molecule per bead. These beads with or without immunocomplexes were loaded and sealed into array of femtoliter sized chambers which were capable of isolating and detecting thousands of single protein molecules captured on the bead via fluorescence imaging [74]. This same approach was also utilized by Song *et al* and his team to evaluate the levels of amyloid beta 1-42 in alzheimer patient's plasma treated with the beta site amyloid precursor protein cleaving enzyme 1 inhibitor. This assay provided the Limit of detection (LOD) and Lower limit of quantification (LLOQ) of 0.3 pg/ml and 2.8 pg/ml respectively.Thus, using SIMOA allows the ultrasensitive detection of various serum proteins associated with various diseases [75].

7. Contact Tracing

Contact tracing is a set of guidelines and monitoring process involved in reducing the spread of infection across the community. It was the most effective step taken in curbing the Ebola outbreak in 2014 – 2016 in various parts of Africa. (World Health Organization Reports) [76]. the people who are tested positive for the COVID 19 infections are contact through contact tracers. The main objective of contact tracer is to make the patient feel comfortable and extract as much as information as possible for an effective contact tracing. Contact tracing is majorly broken down into 3 steps,

1) Contact Identification – Contact identification is the first and foremost step in contact tracing. The contacts which were with the infected person is identified by asking the patient about his personal activates and the role played by the people around them at the onset of illness. The contact can be any people such as family members, friend, colleagues, neighbours or service providers. It's their duty to ensure if the contact feels well, it's better to ask about their current symptoms and ensure that they are safe. The contact tracer should be mature enough to understand the mind-set of the patient and make him understand about the current situation. Contact tracers should also be efficient in convincing the patient that they should isolate themselves to prevent the spread of disease. They should also be in position to give solutions for the access to basic necessities of patients during the isolation period. For example if the patient is living alone and has no one else to support them for food supply, the contact tracers can help them contact a NGO regarding the necessity.

The contact tracers should avoid getting emotionally involved with the patient and mandatory avoid the exposure of the obtained personal information about the patient.

2) Contact Listing: A list of the contacts are made who are found to be in contact with the infected person. Maximum efforts has to be made in the identification of the every individuals. It is essential to inform them about the contact status and the follow up actions they have to take to avoid the community spread.

3) Contact Follow-up: The contacts are called back at regular interval of time to check if their health is fine or medical attention is needed. It also ensures that the person is in isolation and the spread of infection is brought under control. A proper and strict contact tracing plays an effective role controlling the spread of COVID 19 pandemic.

8. Conclusion

Past year since SARS-CoV-2 infection caused a wide spread panic and pandemic all over the world. Several scientists have been striving very hard on developing a vaccine and several research groups are also involved in designing of new test kits. Day by day the rate of infection has been increasing in the world and there is an immediate requirement of kits which are highly accurate and rapid in nature. The future goal of developing an efficient kit involves an onsite test kits which can be developed in mass quantities at most cost effective manner. Development of self-test COVID 19 kits are not far from reach and it will be most useful in proper isolation and quarantine which can be carried out by the individual. By this approach in developing kits, use of expensive laboratory equipments shall be avoided and it saves a lot of time in processing of the sample. The current penned down report provides a broad knowledge on the existing serological and genetic assays which can be used for the detection of COVID – 19 infection. All over the world RT-qPCR dominates in the methodology for the detection of the infection, the other eminent nucleic acid testing strategies such as microarrays, isothermal amplification, cutting - edge CRISPR technology and amplicon based metagenomics approaches are in the final stage of development and much efficient tools can be expected in near future. The serological and immunological testing gives us a deep insight in the hidden information about the role of immune response and durability of the immunity in the population. The test results helps the policy makers and the health care workers to assess the pandemic situation in the right manner so that the normal activities can be resumed at a much faster pace. To conclude the review we can strongly argue that significant progress has been made in the process of developing diagnostic tools. Globally a lot of scientist are working tirelessly for enabling the development of the best kits for detection and a vaccine to prevent the infection. Global collaborations in research work, free flow of knowledge, investments of appropriate funds provides a greater opportunity for creating a safe & secure future.

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Conflict of Interest

The authors declare no conflict of interest.

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