Recent Biotechnological Tools for Diagnosis of COVID-19 Disease: A review

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Abstract

Recently, a corona virus disease (COVID-19) caused by a novel corona virus (Sevier Acute Respiratory Syndrome Corona Virus 2; SARS-CoV-2), rapidly spread throughout the world. It has been resulted an unprecedented public health crisis and has become a global threat. WHO declared it as a pandemic due to rapid transmission and severity of the disease. According to WHO, as of 22nd of August 2020, the disease spread over 213 countries of the world having 22, 812, 491 confirmed cases and 795, 132 deaths recorded worldwide. In the absence of suitable antiviral drugs and vaccines the current pandemic has created an urgent need for accurate diagnostic tools that would be helpful for early detection of the patients. Many tests including classical and high throughput techniques have developed and obtained U.S. Food and drug administration (FDA) approval. However, efforts are being made to develop new diagnostic tools for detection of the disease. Several molecular diagnostic tests such as Real Time-PCR, Real time Isothermal loop mediated amplification (RT-LAMP), full genome analysis by next-generation sequencing (NGS), clustered regularly interspaced short palindromic repeats (CRISPR) technique and microarray-based assays along with other techniques such as Computed Tomography **(**CT) scan, biomarkers, biosensor, nanotechnology, serological test**,** enzyme-linked immunosorbent assay (ELISA), isolation of viral strain in cell culture are currently available for diagnosis of COVID-19 infection. This review provides a brief overview of promising high throughput techniques currently used for detection of SARS-CoV-2, along with their scope and limitations that may be used for effective control of the disease.

Key words: SARS-CoV-2, NAAT, biosensor, CRISPR, ELISA

Conflict of Interest

The authors declare no potential conflict of interest

1. Introduction

Emergence of viral diseases represents a serious threat to global public health. The past few decades have witnessed several viral epidemics that have emerged with increasing frequency, including the severe acute respiratory syndrome corona virus (SARS-CoV identified in 2002/2003), Swine flu (H1N1 influenza identified

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in 2009) and later the Middle East Respiratory Syndrome Corona Virus (MERS-CoV identified in 2012). The latest outbreak of a new corona virus disease known as COVID-19, caused by severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) is highly transmittable and pathogenic viral infection which has spread all over the world. Corona viruses are known to cause disease in humans, other mammals, and birds. Many corona virus species have been identified so far, out of which only six species have been known to cause disease in human being. They were named as HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, SARS-CoV and MERS-CoV [1, 2]. Out of six above identified corona virus species, the first four are endemic locally and causes mild symptoms in humans. Other two can cause severe illness[3]. In late December, 2019, a new viral respiratory illness appeared and spread very fast all over the world which has been the focus of global attention due to a pneumonia epidemic of unknown cause. Initially, the virus was tentatively named as 2019 novel corona virus (2019-nCoV). However, the virus has now been named SARS-CoV-2 by International Committee of Taxonomy of Viruses (ICTV)[4]. This new virus, 2019-nCoV belongs to subgenus Sarbeco virus of the genus beta corona virus of the family Coronaviridae. Viruses belong to the family Coronaviridae possess a single strand, positive-sense RNA genome whose length ranges from 26 to 32 kb. SARS-Cov-2 is genetically more related to SARS-CoV than MERS-CoV, but both are beta-corona viruses believed to be originated from bats. Next-generation sequencing and phylogenetic analysis of the genome revealed 2019-nCoV was closely related (88% identical) to two bat-derived SARS-like corona viruses and more distant from SARS-CoV (79%) and MERS-CoV (50%). At the amino acid level, the 2019-nCoV is quite similar to that of SARS-CoV, but there are some notable differences. For example, the 8a protein present in SARS-CoV are absent in 2019-nCoV; the 8b protein is 84 amino acids in SARS-CoV, but longer in 2019- nCoV, with 121 amino acids; the 3b protein is 154 amino acids in SARS-CoV, but shorter in 2019-nCoV, with only 22 amino acids. Like SARS-CoV and MERS-CoV, SARS-CoV-2 can lead to lethal pneumonia. It also has a stronger human-to-human transmission capacity than SARS-CoV and MERS-CoV[5, 6]. The COVID-19 disease primarily spreads through close contact of respiratory droplets generated by infected individuals [7]. The clinical manifestations of COVID-19 exhibit a range of, symptoms from mild flu to life-threatening conditions. **Most** of the infected patients have reported a high fever while some showed dyspnoea and invasive lesions in both lungs with chest radiographs. However, patients, mostly the younger people those are majority of the workforce and are more likely to be socially active are infected with SARS-CoV-2 could have mild or even asymptomatic and can transmit the virus to others. This might be crucial in further spreading of the disease. Early recognition of an infected person and cutting off the route of transmission are key points to control COVID-19. However, most asymptomatic infections do not seek medical assistance due to no obvious clinical signs and poor prevention awareness, which contribute to the rapid spread of COVID-19. Therefore, it is a great challenge to prevent and control this specific type of patient globally, which requires more attention worldwide.

COVID-19 has become a major global public health concern that has spread very fast with significant morbidity and mortality around the world. As the disease spread to many countries, the World Health

Organisation (WHO) declared the outbreak to be a public health emergency of international concern on 30 January, 2020 and subsequently on March 11, 2020 declared the rapidly spreading corona virus outbreak as a pandemic. As per the World Health Organisation, corona virus disease (COVID-19) situation report– 147), globally, as of 22nd of August 2020, the disease spread over 213 countries of the world having 22, 812, 491 confirmed cases and 795, 132 deaths recorded worldwide.

Early detection with fast and sensitive assays and timely intervention are crucial for interrupting the spread of the COVID-19 virus (SARS-CoV-2)[8]. In the absence of suitable antiviral drugs or vaccines, a reliable detection method of the SARS-CoV-2 infection is critical not only for the prevention and control of the COVID-19 pandemic, but also for clinical treatment. Many countries throughout the world were employing combinations of containment and mitigation strategies, for early diagnosis of COVID-19. Effective strategies need to be implemented in the right time to prevent the spread of disease within the population. Apart from this, early diagnosis is essential for prompt intervention for patients who are at higher risk and there is possibility of developing serious complications while infected with COVID-19. At present several diagnosis methods are available for early detection of the virus and the disease with their advantage and disadvantage (Table-1). However, the successful detection of disease depends on several factors, such as the time of testing from onset of illness, the concentration of virus in the in the sample, the quality of the specimen collected from a person, how it is processed, and the precise formulation of the reagents in the test kits[9].

Till now, the available diagnosis methods for COVID-19 tests are broadly categorized into two types molecular and serological tests. The first category i.e the molecular assays for detection of SARS-CoV-2 viral RNA, uses the reverse transcription-polymerase chain reaction (RT-PCR) based method. However, other methods such as isothermal nucleic acid amplification assays, including transcription-mediated amplification and CRISPR-based methodologies, are some promising alternatives. According to the SARS-CoV-2 structure **(Figure 1)** various genes like E, N, S, ORF and RdRp are targeted for molecular detection. Besides, viral genomic sequencing, a sophisticated technique has become an essential tool for determining the rate and degree of mutational variability associated with SARS-CoV-2. This technique can also be employed for identifying newly emerging strains of the virus for more effective vaccine development.

The second category of diagnostic method includes serological and immunological assays. These serological assays largely rely on detecting antibodies produced by individuals as a result of exposure to the virus while immunological methods are based on detection of antigenic proteins in infected individuals. Detection of antibodies in response to the SARS-CoV-2 virus infection through serological tests can be done through enzyme-linked immunosorbent assay (ELISA) and lateral flow immunoassay. It is important that, these diagnostic techniques can serve for the purposes of detection and management of the COVID-19 pandemic. Testing for SARS-CoV-2 viral RNA by molecular methods identifies SARS-CoV-2-infected individuals during the acute phase of infection as well as to conduct contact tracing for management of disease. On the other hand, antibody based serological testing subsequently identifies individuals who have developed antibodies to the

virus and could be potential convalescent plasma donors. Besides, this antibody base test can be useful to monitor the immune status of individuals and groups exposed to virus over time[10].

Figure 1. Schematic diagram of SARS-CoV-2

In the present scenario the race is going on to develop cost-effective point-of-contact test kits and efficient laboratory techniques for quick diagnosis of SARS-CoV-2 infection. In the present review, a brief discussion on different diagnostic technologies has been discussed with their scope and limitations. This review also provides an overview of current development in COVID-19 diagnostic techniques and products to facilitate future improvement and innovation.

2. Molecular methods for SARS-CoV-2 diagnosis

Now-a-days in viral diagnosis, use of molecular detection methods have become the most sought technology due to their high specificity, faster turnaround times, and absence of limitations posed by the need for susceptible cell lines[11]. For virus detection polymerase chain reaction (PCR) based methods are most routinely applied in molecular biology which commonly multiplies certain portions of genomic material deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) necessary for virus identification. Various recent molecular detection methods that are used for detection of SARS-CoV-2 include nuclei acid amplification test (NAAT), Isothermal nucleic acid amplification, CRISPR technique, viral sequencing and Next generation sequencing approaches which are described below.

2.1. Nucleic Acid Amplification Tests (NAAT) by RT-PCR

SARS-CoV-2 nucleic acid amplification tests (NAATs) were developed very quickly for clinical testing of infections in patients in the early days of Covid-19 pandemic. The genome of corona viruses consists of ribonucleic acid (RNA) rather than DNA. Therefore, a standard PCR cannot be used to detect the presence of the virus. Real-Time Quantitative Reverse Transcription PCR (qRT-PCR) is a major development of PCR technology that provides fast and high-throughput detection and quantification of target DNA sequences.

The target sequences for SARS-CoV-2 specific gene are various genomic region of the virus including the orf1ab and orf1a gene located at the 5'-terminus of the genome that encodes the pp1ab and pp1a proteins, respectively. The 3'-terminus of the genome contains four structural gene such as spike protein (S gene), envelope protein (E genes), membrane protein (M gene) and nucleo capsid protein (N gene). Beside four structural gene it also contain eight accessory proteins (3a, 3b, p6, 7a, 7b, 8b, 9b, and orf14) **(Figure 2)** [5].

Figure 2. Relative positions of qRT-PCR primer-probe set on the SARS-CoV-2 (Slight modification to Jung et al.[12]), Numbers below amplicon are genome positions. Orf1: open reading frame 1; RdRp: RNA-dependent RNA polymerase gene; S: spike protein gene; E: envelop protein gene, N: nucleocapsid protein gene.

The RT-PCR method is aimed to diagnose COVID-19 mainly by targeting these viral genomes (envelope (E), nucleocapsid (N), spike (S), and RNA-dependent RNA polymerase (RdRp) genes) [13-15]. Jung et al.[12] reported various primer and probe sets for SARS-CoV-2. These primers are designed to target different sections of the virus genetic sequence including the envelope E gene, RdRp gene, and the N gene [9, 12, 14, 16]. Among the different genes, targeting the E gene has been reported for highest sensitivity, followed by the RdRp gene for confirmation[16].

In the RT-PCR based technique, RNA is extracted from the upper respiratory tract using the nasopharyngeal swab technique (nose or throat) – where by a sample is taken from the throat behind the nose, containing a mixture of mucous and saliva. Viral Ribonucleic Acid (RNA) is isolated from the swab using laboraty protocol. Usually, the sample is treated with several chemical solutions which help to remove substances, such as proteins and fats, and extracts only the RNA present in the sample.

The RNA is then converted into a complementary strand of Deoxyribonucleic Acid (cDNA) by RNA-dependent DNA polymerase (reverse transcriptase) in a RT-PCR machine. The amplification of DNA is monitored in real time as the PCR reaction progresses **(Figure 3)** and it encompasses a two-step method, typically comprising two enzymes. The first step uses a RNA-dependent DNA polymerase, also known as a reverse transcriptase, to copy RNA into DNA (cDNA), and then the second step switches to the use of Taq polymerase, which amplifies the cDNA as in a standard PCR test. An automated system in the PCR then repeats the amplification process for about 40 cycles. The amplified viral cDNA is then detected, usually by a fluorescent or electrical signal. Thus, RT-PCR is typically very accurate and is generally considered the 'gold standard' of diagnostics. It typically takes 4-6 hours for the test to be completed but with the shipment of samples the turnaround time is 24 hours at best. RT-PCR is capable of analyzing thousands of specimens in a single day and shows a testing sensitivity of 95%[16].

Although RT-PCR is the most widely used method for detecting SARS-CoV-2 infections, it has the limitations. It requires expensive laboratory instrumentation, highly skilled laboratory personnel, and can take days to generate results. The PCR techniques are laborious and expensive as well. Besides, PCR can only detect virus if it is present in sufficient quantity in a person and false negative results have still been reported due to insufficient organisms in the specimen resulting from improper collection, transportation, storage and handling, as well as laboratory test conditions (reagent quality) and personnel operation[17-19]. Further, in RT-PCR clinicians cannot rule out SARS-CoV-2 infection by ruling in other respiratory pathogens among COVID-19 patients. It is important that, recognizing the possible pathogens causing co-infection among COVID-19 patients would help recommendation of appropriate antimicrobial therapy. In this context, the application of multiplexing technology in NAAT has been shown to be very useful in the sense that, it allows better detection than classical methods used for a broad range of viruses. In case of multiplex PCR, more than one target sequence can be amplified by using more than one pair of primers in the reaction. More importantly, multiplex PCR has the potential to save time and effort within the laboratory without compromising test utility[15].

Figure 3. Schematic representation of RT-PCR for amplification of SARS CoV-2 ds DNA

2.2 Droplet digital PCR (ddPCR)

The droplet digital polymerase chain reaction (ddPCR), is a modification of polymerase chain reaction methods. The principle of ddPCR is to divide a traditional PCR reaction mixture, which is similar to the Taqman assay, into smaller reaction system either by diluting to microwell plates, capillaries or oil emulsion[20]. Then these small reactions are run individually. After the run, positive reactions are checked and counted among all reactions. Using Poisson's law of small numbers, the number of templates is positively correlated to the positive wells, so the exact copy number of template can be calculated [21]. It is a third generation of polymerase chain reaction is now widely used in low-abundance nucleic acid detection and is useful in diagnosis of infectious diseases. ddPCR is used for detection of SARS-Cov-2 as complementary to qRT-PCR because it can accurately detect the virus even in very low viral load samples and largely reduce the false negative results, resulted due to qRT-PCR[22]. In an experiment it has been observed that 26 patients those were detected negative by RTCR were fond positive after repeating the experiment in ddPCR[23]. However, ddPCR has some disadvantage. It is currently costlier than qPCR per test with specialized instrumentation and consumables[24].

2.3. Nested RT-PCR

Recently Wang et al. [25] reported a novel locked nucleic acid (LNA)-based one-step single-tube nested real-time qRT-PCR (OSN-qRT-PCR) for detection of SARS-CoV-2 ORF1ab and N genes. Nested reverse transcription polymerase chain reaction (Nested RT PCR) is a modification of reverse transcription polymerase chain reaction used to reduce non-specific binding in products due to the amplification of unexpected primer binding sites. The advantage of this technique is that it can detect the target with low viral load. In this method an aliquot of the product from the primary RT-PCR are taken as a template for a secondary round of PCR amplification. Further amplification of primer-dimer artifacts or nonspecific products generated during the primary PCR are avoided by using different primer sets in the secondary PCR [26]. These "nested" primers are internal to the primers used in the primary PCR, yielding a somewhat shorter amplicon. As their sequences are different from the primary set of primers, hence they will not amplify any nonspecific products generated in the primary PCR. Hence, product specificity is maintained over the high number of amplification cycles combined in the primary and secondary PCRs [26].

The sensitivity of this technique is 10 fold more than the conventional RT-PCR method. In a pilot experiment it has been observed that out of the181 clinical samples tested 14 samples which were given negative result in qRT-PCR test, found positive in OSN-qRT-PCR test [25]. Hence it is confirmed that in compare to RT-PCR the sensitivity of the OSN-qRT-PCR is higher and can be easily used by the clinicians for the diagnosis of SARS-CoV-2 in the early stage of disease when the viral load is very low in patient[27]. However, the lid opening after the first round of nested PCR can increases the risk of cross-contamination which may lead to false positive results [28].

2.4. Isothermal Nucleic Acid Amplification

In contrast to conventional PCR technique, isothermal amplification of DNA/RNA does not require thermal cycling. This technique enables primer binding followed by amplification using a polymerase with strand-displacement activity that separates the strand that is annealed to the target sequence for detection, hence considered as the fastest available molecular laboratory and point-of-care test for the diagnosis of novel SARS-CoV-2.

2.4.1. Loop-Mediated Isothermal Amplification (LAMP)

Instead of using a series of temperature changes like in conventional RT PCR, LAMP technique based on synthesis of target DNA at constant temperature of 60–65 ^{0}C using specially designed primers and enzyme (DNA polymerase). It operates with strand displacement activity instead of heat denaturation as in other RT-PCR techniques[29, 30]. For strand-displacement activity, LAMP uses a *Bst* DNA polymerase, with two inner (FIP, BIP), and outer (F3, B3) primers. These primers recognise six different regions on the target DNA. Besides, additional Loop-primers (Forward loop primer LF, and backward loop primer; LB) can be used to speed up the reaction by binding and amplifying newly formed loop amplicons in the reaction. The role of these additional primers is to further enhance the sensitivity and specificity of the reaction[31]. The mechanism of LAMP technology involves three steps i.e, an initial step, a cycling amplification step and an elongation step **(Figure 4)** [32]. In the first step, the forward inner primer (FIP) anneals to the initial template DNA at the F2c sequence and carry out the extension reaction by the help of the enzyme *Bst* polymerase that produces the complementary DNA strand. Immediately after the FIP (BIP) primer, two other primers such as F3 and B3 bind and displace the newly synthesized DNA strand, simultaneously releasing the target DNA or FIP (BIP)-linked complementary DNA strand. *Bst* polymerase then replaces the F3 site of target DNA sequence with F1c of newly released single strand and forms the initial stem loop-loop structure. Simultaneously, BIP and B3 primers bind to this DNA and subsequently, an extension reaction occurs resulting in the formation of single-stranded dumbbell-like structure with loops at both ends. The formation of the dumbbell-like product is essential for LAMP to establish isothermal amplification. This is because the loop structures are always single stranded and can be annealed by FIP or BIP. Thus, it is found that, the formation of the loop structure can lead to the elimination of the denaturing step, which is otherwise essential in PCR for obtaining single-stranded DNA. After the formation of the dumbbell-like structure, a cyclic reaction is spontaneously established for further amplification in the LAMP reaction along with production of various copies of the target sequences. After amplification, the viral DNA can be detected with naked eye when the reaction mixture is turned turbid due to production and precipitation of magnesium pyrophosphate in reaction mixture. The turbidity or cloudiness can be measured via photometry. Since real-time RT-LAMP diagnostic testing requires only heating and the cloudiness can be seen by the naked eye, it becomes a promising tools for COVID-19 virus detection by scientists and clinicians. The ID NOW COVID-19 test, is one of the best real time RT-LAMP technology available for molecular detection of SARS-CoV-2.

Lu et al. [33] using a mismatch-tolerant amplification technique, developed a RT-LAMP assay for SARS-CoV-2 detection based on its N gene. Its results can be monitored within 30 min using a real-time PCR machine

or visualized via colorimetric change within 40 min from red to yellow **(Figure 5a)** when the template input is more than 200 copies per 25 µL reaction. To perform the LAMP test specifically for COVID-19, Song et al [34] selected conserved COVID-19 sequences using Clustal X and further designed LAMP primers. In order to make the detection process simpler, they used leucocrystal violet (LCV) dye as a chromogenic reagent, providing an obvious, deep violet color change that could be observed with the naked eye. The entire diagnostic process was relatively simple and required only a single tube for reaction.

Similarly, El-Tholoth et al. [35] included leucocrystal violet (LCV) in the LAMP reaction mix in Closed-Tube Penn-RAMP assay. The LCV dye is nearly colorless in the absence of dsDNA and deep violet in presence of dsDNA, enabling detection of amplicons. **(Figure 5b)**

Figure 4. Loop-Mediated Isothermal Amplification

Figure 5. Visual detection of SARS-CoV-2 in RT-LAMP diagnostic method via colorimetric change from (a) from red (negative) to yellow (positive) (Lu et al. 2020). (b) from lighter (negative) to dark (positive) [35]. (Sorce: Kashir and Yaqinuddin [36]) (Free Copyright permissions granted for by Elsevier Ltd. for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source as long as the COVID-19 resource centre active)

Recombinase polymerase amplification (RPA) is another approach that integrates isothermal methods for reverse transcription followed by recombinase activity that mediates primer (targeting the N gene) binding to the homologous sequence in dsDNA. Subsequent amplification by polymerase mediated primer extension achieved 100% diagnostic sensitivity and specificity. This method offers potential advantages over RT-PCR for speed, scale and portability, allowing evidence-based clinical decisions to be made during a patient visit. This method detected total viral RNA derived from cell culture supernatant for SARS-CoV-2 [37].

2.4.2. Penn RAMP technology

At the in the initial stage of infection, the amount of viral samples is very low, it is sometimes very difficult to detect it by RT-PCR which gives false-negative result[18]. To improve COVID-19 diagnostic test sensitivity, Song et al [34] at the University of Pennsylvania discovered a two-stage isothermal dsDNA amplification method which can use both recombinase polymerase amplification (RPA) and loop-mediated isothermal amplification (LAMP) techniques in a single tube known as closed-tube Penn-RAMP technology This modified LAMP assay are used for the rapid diagnosis of COVID-19 and 10 times higher sensitive than LAMP and RT-PCR method [35].

Nasal sample are collected with swab, and eluted in warm water at above 65 °C. After elution, the samples are inserted into a tube for amplification. The incubation reactions are carried out by two isothermal amplification stages. Hence it is known as two stage Penn-RAMP process. At the first stage for Recombinase Polymerase Amplification (RPA) are carried out at 38 °C in the cap of the tube using the outer LAMP primers F3 and B3 with various target concentrations to amplify all targets. LAMP is carried out in the second stage at 63 °C in the same tube with the rest 4 primers used in LAMP but without any target. The ratio of the RPA to LAMP reaction mixtures should be at 1: 9. After loading the tube cap with RPA mix and the tube itself with the LAMP mix, the tube are sealed and remained so throughout the duration of the process. The closed tube was incubated at 38 °C for15-20 min in a thermal cycler and after incubation, a short spin or flipped back and forth a few times to mix the RPA and LAMP reaction mixture. The reaction mixtures are then incubated in a thermal cycler at 63°C for 40 min with real time signal monitoring [35]. **(Figure 6)**

Figure 6. Schematic representation of Penn-RAMP procedure [35]. (Sorce: Kashir and Yaqinuddin [36]) (Free Copyright permissions granted for by Elsevier Ltd. for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source as long as the COVID-19 resource centre active)

2.5. CRISPR (Clustered regularly interspaced short palindromic repeats) technology

Another recent technolog**y** that can detect the SARS-CoV-2 RNA is clustered regularly interspaced short palindromic repeats (CRISPR)-Cas-system. An engineered type II CRISPR-Cas9 system consists of a Cas9 endonuclease and a single guide RNA (sgRNA). The Cas9 endonuclease becomes active after binding with sgRNA and guided by the sgRNA to attach at a specific site of the target DNA. After attachment the Cas9 endonuclease cleaves both the strand of the target DNA [38]. Instead of Cas9 endonuclease, Cas12 or Cas13 endonuclease are recently programmed to target and cut SARS-CoV-2 viral RNA sequences [39].

SHERLOCK (Specific High Sensitivity Enzymatic Reporter UnLOCKing) is a detection strategy that uses Cas13a ribonuclease for cleavage of COVID-19 S gene and Orf1ab gene in RNA fragments. In SHERLOCK detection technique, viral RNA are collected from nasopharyngeal swab/ **t**hroat of swab samples of patient and reverse transcribed to cDNA and isothermally amplified using reverse polymerase amplification [40, 41] **(Figure 7a)**. The amplified products are transcribed back into RNA. Cas13a complexes with a RNA guide sequence binds to that amplified RNA product [42]. Upon binding the target, Cas13a is activated and cleaves it along with the surrounding fluorophore-quencher probes which produce a fluorescent signal [40, 41]. In addition to SHERLOCK, Broughton et al. [43] reported use of CRISPR cas 12 system for detection of SARS-CoV-2 known as SARS-CoV-2 DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR) technique. In this method RNA extracted from nasopharyngeal or oropharyngeal swabs in universal transport medium (UTM). Reverse transcription and isothermal amplification of the extracted viral RNA are carried out using loop-mediated

amplification (RT–LAMP) followed by Cas12 detection of predefined corona virus sequences (E and N genes of viral RNA), after which cleavage of a reporter molecule confirms detection of the virus [43] **(Figure 7b)**.

Figure 7. Schematic representation of (a) nuclease activity of Cas1 3a, activated upon specific binding of gRNA to the *Orf1ab* gene. Fluorescent signal produced from cleaved probes is captured and indicates the presence of 2019-nCoV (With prior copyright permission Hou, et al. [41]). (b) Schematic of SARS-CoV-2 DETECTOR workflow. Conventional RNA extraction can be used as an input to DETECTOR (LAMP preamplification and Cas12-based detection for E gene, N gene and RNase P), which is visualized by a fluorescent reader (Slight modification to Broughton et al. [43]).

SHERLOCK are now used in point of care testing contexts, after its modification called STOP (SHERLOCK Testing in One Pot) covid-19 test. This STOP COVID-19 test are performed at a single temperature with one fluid handling step and does not require RNA extraction. In this technique, sample extracted from nasopharyngeal swab or saliva is mixed with the lysis buffer. After mixing with the lysis buffer the mixture is transferred to negative control and test SHERLOCK reactions and heated for 60 mins at 60 °C. After 60 mins a detection strips are dipped into each reaction for 2 minutes for lateral flow readout **(Figure 8)** [44]. Results appear on an easy-to-read strip that is akin to a pregnancy test, in the absence of any expensive or specialized lab equipment. Moreover, the researchers spiked mock SARS-CoV-2 genomes into healthy saliva samples and showed that STOP Covid is capable of sensitive detection from saliva, which would obviate the need for swabs in short supply and potentially make sampling much easier. The test aims to ultimately be simple enough that anyone can operate it in low-resource settings, including in clinics, pharmacies, or workplaces, and it could potentially even be put into a turn-key format for use at home [44].

Figure 8. Steps of the one-step STOP Covid test (With prior copyright permission from Joung et al. [44])

2.6. Microarray.

Due to its specificity, microarray assays is one of the promising alternative for diagnosis of SARS-CoV-2 [45]. Viral mRNA is extracted from the nasopharyngeal swab of the patient and converted to cDNA and labelled with a specific florescent probe. Then the labelled florescent probe cDNA is hybridised onto a DNA microarray where it will hybridize to their synthetic complementary DNA probes attached on the microarray. If they hybridize strongly, then they will remain attached after washing. The fluorescent tags on bound cDNA are excited by a laser and the fluorescently labelled target SARS COV-2 specific nucleic acid sequences that bind to a probe generate a signal [45]. This technique is proven to be useful for the detection of any mutations that associated with SARS-CoV-2 spike (S) gene through single nucleotide polymorphisms (SNP) [46].

2.7. Viral sequencing

Detection of SARS-CoV-2 genome sequence through Genomic sequencing is very useful tools which help to get the information about viral mutations, evolution, molecular epidemiology of the disease and thus can help in the development of new therapies and vaccines [47]. Viral RNA are extracted from the nasopharyngeal swab of the patient and further purified to remove human cytoplasmic and ribosomal rRNA. The purified viral RNA is reverse transcripted into cDNA and amplified by highly specific, universal CoV primers. The DNA amplicons are then sequenced to detect the similarity with the SARS-CoV-2 typing. In the recent years several SARCOV-2 sequences are deposited into the Global initiative on sharing all influenza data (GISAID), NCBI GenBank and China National GeneBank DataBase (CNGBdb) [47] and to date, there are over 17,000 SARS-CoV-2 sequences from global have been submitted.

A high-throughput sequencing technique along with bioinformatics tools known as Next-generation sequencing (NGS) is an important sequencing technique for identifying unknown pathogens, and mutation or recombination in the genome very quickly [48]. Through this technique it was possible to sequence the complete genome (30,000 nucleotides) of the SARS-CoV-2. After the successful complete genome sequencing it could be identified as a new strain of an RNA virus which belonged to the Coronaviridae family and was named as SARS-CoV-2 [49]. NGS not only helped for identification of SARS-CoV-2 but also, provides insight into strain origin and viral evolution.

3. **Serological test**

Serological surveys were important for investigation of an ongoing outbreak and retrospective assessment of the attack rate or extent of an outbreak. In cases where NAAT assays are negative and there is a strong epidemiological link to COVID-19 infection, paired serum samples could support diagnosis once validated serology tests are available. Serum samples can be stored for these purposes. Cross reactivity to other corona viruses can be challenging [50]. But commercial and non-commercial serological tests are currently under development. Some studies with COVID-19 serological data on clinical samples have been published [51, 52]. Serological tests detect the presence of the body's immune response to a pathogen. Antibodies, also known as immunoglobulins (Ig), are produced by B cells and are part of a highly specific defence against new antigens [53]. Two classes of antibodies, immunoglobulin M (IgM) and immunoglobulin G (IgG), are common targets for serological tests because of their roles in targeting and destroying new infections. The immune system typically produces IgM soon after infection as a frontline defence, and IgG is generated later. Additionally, IgG persists in the body longer than IgM and contributes to longer-term immune memory, which enables the immune system to rapidly identify and respond to future infections by the same pathogen. IgA is another type of antibody, typically found in mucous membranes that can be produced in high quantities during infections [54]. Current evidence indicates that these antibodies begin to develop approximately 6 to 10 days after infection with SARS-CoV-2 [55]. IgM appears to peak approximately 12 days after SARS-CoV-2 infection and persists in sufficient

quantities for as long as 35 days, after which the quantity declines rapidly [56]. IgG has been observed to peak approximately 17 days after SARS-CoV-2 infection and persist for at least 49 days (at which time the study was concluded) [54]. Further, IgG has been observed in patients 2 weeks after symptom onset [57]. IgG has been found in patients up to 2 years after recovery from severe acute respiratory distress syndrome (SARS) [58]. Thus, IgM can be an indicator of early stage infection, and IgG can be an indicator of current or prior infection. IgG may also be used to suggest the presence of post-infection immunity [39]. 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, the spike glycoprotein and nucleocapsid protein. In the context of SARS-CoV-2, rapid diagnostic tests (RDTs), enzyme-linked immunosorbent assays (ELISAs) and neutralization assay have been designed to detect antibodies specific to the spike (S), nucleocapsid (N), membrane (M), and envelope (E) proteins of the SARS-CoV-2 virus

3.1. Rapid diagnostic tests for antigen detection

Antigen (Ag) is a foreign molecule present at the pathogen which can bind to antigen-specific antibody (Ab) or B cell antigen receptor (BCR). To detect the antigen, first it is very important to know the antigenic structure of SARS-CoV-2. The structure of SARS-CoV-2 comprises of spikes, which are formed by the spike protein (SP) **(Figure 1)**. SP is a major glycoprotein (Mol. Wt. ~180 kDa) that consists of two subunits, i.e, S1 and S2 [59]. S1 contains a receptor binding domain (RBD), which is responsible for recognizing and binding with the host cell receptor, i.e, angiotensin converting enzyme 2 receptor (ACE2) found in the lower respiratory tract [60]. On the other hand, S2 contains other basic elements needed for the membrane fusion. SP is the common target for neutralizing antibodies and vaccines, while the amino-terminal S1 subunit of SP is the most variable immunogenic antigen. Additionally, SARS-CoV-2 has nucleus protein (NP) (Mol. Wt. ~40 kDa), the most abundant viral phosphoprotein produced and shed during infection. NP exhibits high immunogenicity and can be detected in either serum or urine samples during the first two weeks of infection with peak viral shedding around ten days after infection [3]. Being a large protein, it can be detected via a sandwich immunoassay. Furthermore, SARS-CoV-2 contains membrane protein (MP), which is the most abundant protein on the complete virion particle. The envelope protein (EP) is the smallest major structural protein of SARS-CoV-2, which is involved in viral assembly, release of virions, and pathogenesis. Several antigenic protein detection methods are developed. Rapid diagnostic test (RDT) detects the presence of viral proteins (antigens) expressed by the COVID-19 virus in a sample from the respiratory tract of a person ([9, 61]). If the target antigen is present in sufficient concentrations in the sample, it will bind to specific antibodies fixed to a paper strip enclosed in a plastic casing and generate a visually detectable signal, typically within 30 minutes. The sensitivity of these tests might be expected to vary from 34% to 80% [62]. On 8 May 2020, the U.S. Food & Drug Administration (FDA) granted the first Emergency authorization for a Sofia SARS Antigen Fluorescent Immunoassay (FIA) technology called Sofia 2 SARS Antigen FIA [63]. The Sofia 2 SARS Antigen FIA technology employs immunofluorescence technology in a sandwich design that uses monoclonal antibodies to detect nucleo capsid (N) protein from SARS-CoV and SARS-CoV-2 but does not differentiate, between the two viruses. The patient

sample is placed in the reagent tube, during which time the virus particles in the sample are disrupted, exposing internal viral nucleoproteins. After disruption, the sample is dispensed into the test cassette sample well. From the sample well, the sample migrates through a test strip containing various unique chemical environments. If SARS-CoV or SARS-CoV-2 viral antigen is present, they will be trapped in a specific location. Sofia 2 will scan the test strip and measure the fluorescent signal by processing the results using method specific algorithms. Sofia 2 will display the test results (Positive, Negative, or Invalid) on the screen. A negative test result may occur if the level of antigen in a sample is below the detection limit of the test and should be confirmed with an RT-PCR test [63].

3.2. Rapid diagnostic test for antibody detection:

Rapid diagnostic test (RDT) for antibody detection relies on a lateral flow assay that returns qualitative (positive or negative) result can be obtained in 10−30 min. Lateral flow tests can detect antibody to virus generated in patient blood indicating that the patient has COVID-19 or has recovered from COVID-19 [61]. However, it cannot distinguish between an active and a previous infection. RDTs are not capable of providing quantitative results indicating the amount of the antibodies in the specimen. They are small, portable, and can be used at point-of-care (POC). These tests provide a hugely important ability to detect past infection with virus to identify people who were asymptomatic, people who have cleared the virus and so no longer risk being infected or spreading the virus to others [30]. In addition, antibody tests are critical for assessing population spread of the virus and the level of 'herd' immunity in the population. This is important for understanding the potential consequences of lifting or enforcing measures to control the virus such as quarantine, social distancing, school and workplace closures. The antibody IgG and IgM lateral flow immunoassay tests are very simple to read: A control line must appear to show that the assay has worked correctly **(Figure 9)**. Then, test lines will appear if either of the antibody types is found in the sample. The appearance of lines for IgG or IgM, or both indicate a positive test – showing that the patient has been infected with the COVID-19 corona virus. For this test, a small blood sample collected either from a vein or from a small finger prick, is placed at one end of the test strip **(Figure 10).** A few drops of a diluting liquid called a 'buffer' are also added to help the blood sample flow across the device. As the sample moves through the device, antibodies against SARS-CoV-2 that are present in the sample will attach to chemicals in the device, capturing the antibodies on the test and control lines. This capturing and binding process results in a colour change along the test and control lines which can be seen by eye, producing one, two or three lines depending on the type of antibodies are present (IgM or IgG) [30].

Figure 9. Validity test of RDT technique [30]

Figure 10. Detection of antibodies by RDT method [30]

3.3. Antibody detection through Enzyme-linked immunosorbent assays (ELISA):

Enzyme-Linked Immunosorbent Assay (ELISA) is a serological test or blood-based test. ELISA is not only a test for positive or negative of the infection, but also gives us a measure of antibodies. This is particularly important for developing a vaccine and also for convalescent plasma therapy for SARS-CoV-2. The test is used to identify whether a person has been exposed to COVID-19 or not. This is done by detecting the presence of immunoglobulin IgM and IgG antibodies. The test relies on antibodies to detect a target viral protein or antigen using highly specific antibody-antigen interactions. The test involves coating viral proteins (like spike protein) on a plate **(Figure 11)**. Patient samples are then applied on viral protein, and if the patient has antibodies to the viral protein they bind together. The bound antibody-antigen can then be detected with another wash of antibodies that produce a colour. It relies on specific binding of COVID-19 patients IgG and IgM antibodies present in whole blood, plasma, or serum samples. For this test, SARS-COV-2 viral protein is coated in a 96-well plate. Diluted human serum is added for 1 h, after which the plate is washed again. Antihuman IgG functionalized 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 will be sandwiched between the adsorbed nucleoprotein and the antihuman IgG probe, resulting in a positive signal [64].

Figure 11. Enzyme-linked immunosorbent assay for COVID-19 antibody detection [30].

3.4. Neutralization assay

The assay provides quantitative information on the ability of patient antibodies to confer protective immunity. It determines the ability of an antibody to inhibit virus infection of cultured cells and the resulting cytopathic effects of viral replication [39]. For this assay, patient samples of whole blood, serum, or plasma are diluted and added at decreasing concentrations to the cell cultures. If neutralizing antibodies are present, their levels can be measured by determining the threshold at which they are able to prevent viral replication in the infected cell cultures [39]. Because these tests require live virus to challenge the antibodies, neutralization assays

must be performed in the appropriate biosafety containment level (Biosafety Level 3, or BSL-3, or above) and require a week or longer to return results. Despite these limitations, determination of neutralizing antibodies is important in the short term for the therapeutic application of convalescent plasma and, in the long term, for vaccine development [39]. Live virus is added to serial dilutions of patient serum and then cultured in a cell line on an agar plate. Over several days, patient antibodies with neutralizing capacity will prevent the virus from creating "plaques," or small areas of no cell growth, on the plates. Researchers can quantify the neutralizing capacity of patient antibodies by comparing the serial dilution plates and the control plates. The development of serologic assays that accurately assess prior infection and immunity to SARS– CoV-2 will be essential for epidemiologic studies, ongoing surveillance, vaccine studies, and potentiality for risk assessment of health care workers. Immunoassays are already on the market in some countries, but their diagnostic accuracy and optimal use remain undined. However, results from antibody testing should not be used as the sole basis to diagnose SARS-CoV-2 infection [65]. False positives can occur due to cross-reactivity with antibodies from previous infections, such as from other coronaviruses. People who test negative should be checked again in a few days, and positive results should be confirmed by other methods [65].

4. Other techniques

4.1. Computed Tomography (CT) Scan

Besides molecular and serological diagnosis, non-contrast chest CT scan is also used for early diagnosis of viral disease. Cross-sectional X-ray images of a patient's chest are taken at different angles and analyzed for any types of abnormalities [66]. Between two days or in the early stages of COVID infection normal CT images are observed [67]. Between 0–4 days after symptom onset, bilateral and peripheral ground-glass opacities (areas of hazy opacity) and consolidations of the lungs (fluid or solid material in compressible lung tissue) are observed [67-69] **(Figure 12)**. But after around 10 days when the symptoms appeared in the patient the COVID-19 virus affects the lungs [69] and CT scan images including sub pleural dominance, crazy paving pattern (*i.e*, irregular-shaped paved stone pattern), followed by increasing consolidation of the lungs may develop as the disease evolves [70, 71]. From comparative study it has been observed that sensitivity of CT scan diagnosis for COVID-19 infection was 86-98% and improved false negative rates compare to the sensitivity of RT-PCR [72, 73]. In contrast**,** the chances of overlapping of CT scans images with other viral pneumonia can't be avoided hence the specificity of CT scan for COVID-19 is very low (25%) and the reason why Chest CT scans are not recommended for routine screening [74].

Figure 12. CT scan images showing patchy consolidations and ground glass opacities in both lungs of a covid-19 patient [70] (With prior copy right permission from the Journal Manager of European Journal of Radiology).

4.2. Biosensor Test.

Biosensor is an integrated analytical device equipped with transducer, bioreceptor and signal detector used to detect chemical substances. An electronic signal is generated when the bioreceptor interact with the target analyte through transduces which can then be further amplified by a detector circuit, processed, and displayed. Recently biosensor is developed that can detect SARS-CoV-2 viruses in the air. Seo et al. [75] reported a Field-effect transistor (FET)-based biosensing device for detecting SARS-CoV-2 in clinical samples **(Figure 13).** This sensor device is very sensitive diagnostic instrument for COVID-19 which doesn't need any labelling or pretreatment of the sample. In this sensor a specific antibody was coated with graphene sheets of the FET that can detect the spike protein of SARS-CoV-2 extracted from nasopharyngeal swab of the COVID-19 patients. Biosensor could in the future be deployed in hospitals, train stations, laboratories and more to combat the spread of the COVID-19 pandemic.

In another report, Mokhtarzadeh et al. [76] discovered a nano-biosensor that can easily enhance the signal due to its tunneling and quantum properties leading to amplification [77]. Advanced nano-biosensors with aptamers specially designed for COVID-19 are another potential biosensor device can be used for rapid diagnosis of SARS-CoV-2 even in person without any symptoms with high sensitivity, specificity and selectivity in a cost effective and user friendly manner as compared to the conventional methods [78].

4.3. Biomarkers

Biomarker is a naturally occurring molecule, gene, or measurable indicator characteristic by which a particular pathological or physiological process, disease, etc. can be identified. In the search for COVID-19 vaccine biomarkers can be a beneficial tool and will help speed up clinical trials. According to Cheng et al. [79] a patient infected with COVID-19 trials observed increased level of C-reactive protein, lactate dehydrogenase, lymphopenia and D-dimer whereas the level of lymphocytes, leukocytes, and blood platelets decreased than the normal and can be considered as Biomarker for COVID-19 diagnosis [80-82]. The disadvantage of using these biomarkers is that they are also abnormal in other illnesses and no such specific biomarker currently discovered that can directly have used for diagnosis of COVID-19 [79]**.**

Figure 13. Field-effect transistor (FET)-based biosensing device for detection of SARS-CoV-2 (Seo et al.[76]). (Sorce: https://pubs.acs.org/doi/10.1021/acsnano.0c02823), (further permission related to the material excerpted should be directed to the ACS).

4.4. Nanotechnology

Moitra et al. [83] developed a colorimetric assay in which gold nanoparticles (AuNPs), capped with thiol modified antisense oligonucleotides (ASOs) specific for N-gene (nucleocapsid phosphoprotein) of SARS-CoV-2, is used for diagnosing positive COVID-19 cases within 10 minutes from the isolated RNA samples. In the presence of targeted RNA sequence of SARSCoV- 2, this thiol modified ASO capped AuNPs, agglomerate and exhibits a change in its surface plasmon resonance (SPR) which is further amplified with the addition of RNaseH. The

RNaseH cleaves the RNA strand from the RNA-DNA hybrid which results in the precipitation of gold nanoparticles (AuNPs) present in the reaction mixture and can be detected visually. The agglomeration of ASO capped gold nanoparticles in the presence of SARS-CoV-2 RNA was also detected by UV-Visible absorbance spectroscopy, transmission electron microscopy, and enhanced dark-field hyperspectral imaging technique. This naked-eye detection technique of SARS-CoV-2, doesn't need any expensive instrumental techniques like other detection method.

Similarly, Zhao et al.[84] reported the viral RNA extraction method using poly (amino ester) carboxyl groups (PC)-coated magnetic nanoparticles (pcMNPs), for the detection of SARS-CoV-2. Due to the strong interaction between carboxyl groups and nucleic acids, the poly carboxyl-functionalized MNPs (pcMNPs) enables rapid and efficient absorption of RNA molecules. This RNA extraction method combines the lysis and binding steps into one step and can purify viral RNA from multiple samples within 20 min. To reduce the operation time and risk of cross contamination this extracted RNA are introduced into subsequent RT-PCR without elution step. By identifying two different regions (*ORFlab* and *N* gene) of viral RNA, 10-copy sensitivity and a strong linear correlation between 10 and 10^5 copies of SARS-CoV-2 pseudovirus particles are achieved. Due to its simplicity, satisfactory performances and robustness, this method provides a promising alternative to decrease the labour-intensity and reduce possibility of false-negative results in current RT-PCR-based SARS-CoV-2 diagnosis.

4.5. Viral Culture

Though general diagnosis of COVID-19 patients, cannot recommended through viral culture but can be used for research analysis especially for identification, detection of mutation if any, analysis of specific properties of the mutated strain and also for development of vaccine. Human airway epithelial cell lines were used for the initial isolation of the virus [85]. Without a host cell no reproduction is possible, so a diagnostic method employing culture of the virus alone is not possible. Culture based diagnostic tolls have been developed in cased by infecting host cells, but such an approach poses a relatively high level of complexity and hence is not ideal.

5. Challenges for Diagnosis

There are several diagnostic challenges associated with the current SARS-CoV-2 outbreak. First, The SARS-CoV-2 is closely related to other important coronavirus subspecies and species, so diagnostic assays can yield false positives if they are not exquisitely specific to SARS-CoV-2. Secondly, suspected SARS-CoV-2 patients sometimes have a different respiratory viral infection or have co-infections with SARS-CoV-2 and other respiratory viruses. Therefore, it is important to characterize these other pathogens, for both patient diagnostics and outbreak response.

The COVID-19 outbreak has had a major impact on clinical microbiology laboratories in the past several months. Early diagnosis of COVID-19 is essential for the timely management as well as isolation of confirmed cases to prevent further transmission of disease. Diagnostic tests for COVID-19 have loomed large in the ongoing coronavirus pandemic as an essential tool to track the spread of the disease. Even though excellent

techniques are available for the diagnosis of symptomatic patients COVID-19 in well-equipped laboratories; critical gaps still remain in screening asymptomatic people who are in the incubation phase of the virus. Furthermore, the sensitivity of the testing kits is a matter of debate and thereby a sizeable number of patients may not be identified, which may ultimately be detrimental in the early diagnosis and treatment of COVID-19 cases. Also in low and middle-income countries (LMIC), the healthcare system is not robust enough as a result of which the testing laboratories often face difficulties in the performance of molecular testing [86].

6. Conclusion

A global outbreak of a new emerging illness, severe acute respiratory syndrome (SARS), associated with a novel corona virus, SARS-CoV-2 has become a pandemic. Rapid laboratory diagnosis of SARS-CoV-2 infection is important for identification of the disease, managing patient care, improving surveillance and preventing nosocomial transmission. Transmission electron microscopy, genome sequencing, RT-PCR tests, isothermal RT LAM amplification, Penn RAMP technology**,** CRISPR technology, Microarray test, serological tests, antigen detection test, CT scan, detection through Biomarker, Biosensor, Nano-techniques and viral culture techniques have been developed and can be rapidly implemented in an outbreak situation. However, development of new test is still needed which should be robust and conductible in the field as well as at local point-of-care (POC) centres, without the requirement of specialized equipment and highly trained professionals to interpret results. Current methods of diagnosis of the novel corona virus mostly rely on identification of particular genetic sequences or antibodies but new assays are required urgently for instant detection of the infection as well as to meet the growing demand for rapid detection.

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References

1. Arabi YM, Balkhy HH, Hayden FG, Bouchama A, Luke T, Baillie JK, Al-Omari A, Hajeer AH, Senga M, Denison MR, Nguyen-Van-Tam JS, Shindo N, Bermingham A, Chappell JM, Kerkhove MDV, Fowler RA. Middle East Respiratory Syndrome. *N Engl J Med.* 2017;376:584–594.
2. Skariyachan S, Challapilli SB, Packirisamy S, Kumargowda ST, Sridhar VS. Recent Aspects on the Pathogenesis Mechanism, Animal Models and Novel Therapeutic Interventions for Middle East Respiratory Syndrome Coronavirus Infections. *Front Microbiol*. 2019;10:569.
3. Paules CI, Marston HD, Fauci AS. Coronavirus infections-More than just the common cold. *JAMA*. 2020; 323:707–708.
4. International Committee on Taxonomy of Viruses (ICTV). Naming the 2019 Coronavirus. 2020. Available at: https://talk.ictvonline.org/

5. Wu A, Peng Y, Huang B, Ding X, Wang X, Niu P, Meng J, Zhu Z, Zhang Z, Wang J, Sheng J, Quan L, Xia Z, Tan W, Cheng G, Jiang T. Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. *Cell Host Microbe*. 2020;27:325e8.
6. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, Graham BS, McLellan JS. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science*. 2020;367:1260–1263.
7. United States Centers for Disease Control and Prevention (CDC). Interim infection prevention and control recommendations for patients with suspected or confirmed coronavirus disease 2019 (COVID-19) in healthcare settings. Coronavirus Disease 2019 (COVID-19). 2020. Available at: https://www.cdc.gov/coronavirus/2019-ncov/hcp/infection-control-recommendations.html).
8. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B, Zhu N, Bi Y, Ma X, Zhan F, Wang L, Hu T, Zhou H, Hu Z, Zhou W, Zhao L, Chen J, Meng Y, Wang J, Lin Y, Yuan J, Xie Z, Ma J, Liu WJ, Wang D, Xu W, Holmes EC, Gao GF, Wu G, Chen W, Shi W, Tan W. Genomi characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet*. 2020;395:565-574.
9. World Health Organization report Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases. 2020a. Available at: (file:///C:/Users/biolab/AppData/Local/Temp/WHO-COVID-19-laboratory-2020.4-eng.pdf)
10. Jones H. The Importance of diagnostic testing for COVID-19. *Infect Dis Hub*. 2020. Available at: https://www.id-hub.com/2020/04/02/the-importance-of-diagnostic-testing-for-covid-19/
11. Fox JD. Nucleic acid amplification tests for detection of respiratory viruses. *J Clin Virol*. 2007;40:S15–S23.
12. Jung YJ, Park GS, Moon JH, Ku K, Beak SH, Kim S, Park EC, Park D, Lee JH, Byeon CW, Lee JJ, Maeng JS, Kim SJ, Kim SI, Kim BT, Lee MJ, Kim HG. Comparative analysis of primer-probe sets for the laboratory confirmation of SARS-CoV-2. *bioRxiv*. 2020;02.25.964775;
13. Binnicker MJ. Emergence of a Novel Coronavirus Disease (COVID-19) and the Importance of Diagnostic Testing: Why Partnership between Clinical Laboratories, Public Health Agencies, and Industry Is Essential to Control the Outbreak. *Clin Chem*. 2020;66(5):664-666.
14. Chu DKW, Pan Y, Cheng SMS, Hui KPY, Krishnan P, Liu Y, Ng DYM, Wan CKC, Yang P, Wang Q, Peiris M, Poon LLM. Molecular Diagnosis of a Novel Coronavirus (2019-nCoV) Causing an Outbreak of Pneumonia. *Clin Chem*. 2020;66(4):549-555.
15. Chan JFW, Kok KH, Zhu Z, Chu H, To KKW, Yuan S, Yuen KY. Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. *Emerg Microbes Infect*. 2020;9:221-236.
16. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DKW, Bleicker T, Brünink S, Schneider J, Schmidt ML, Mulders DGJC, Haagmans BL, van der Veer B, van den Brink S, Wijsman L, Goderski G, Romette JL, Ellis J, Zambon M, Peiris M, Goossens H, Reusken C, Koopmans MPG, Drosten C.

Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill*. 2020;25(3):2000045.

17. Han Y, Yang H. The transmission and diagnosis of 2019 novel coronavirus infection disease (COVID-19): A Chinese perspective. *J Med Virol.* 2020;92:639-644.
18. Xie C, Jiang L, Huang G, Pu H, Gong B, Lin H, Ma S, Chen X, Long B, Si G, Yu H, Jiang L, Yang X, Shi Y, Yang Z. Comparison of different samples for 2019 novel coronavirus detection by nucleic acid amplification tests. *Int J Infect Dis*. 2020;93:264-267.
19. Zou L, Ruan F, Huang M, Liang L, Huang H, Hong Z, Yu J, Kang M, Song Y, Xia J, Guo Q, Song T, He J, Yen HL, Peiris M, Wu J. SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients. *N Engl J Med*. 2020;382:1177-1179.
20. Rougemont M, Saanen MV, Sahli R, Hinrikson HP, Bille J, Jaton K. Detection of four plasmodium species in blood from humans by 18s rrna gene subunit-based and species-specific real-time PCR assays. *J Clin Microbiol.* 2004;42:5636-5643.
21. Li H, Bai R, Zhao Z, Tao L, Ma M, Ji Z, Jian M, Ding Z, Dai X, Bao F, Liu A. Application of droplet digital PCR to detect the pathogens of infectious diseases *Biosci Rep.* 2018;38:1-8.
22. Hindson BJ, Ness KD, Masquelier DA, Belgrader P, Heredia NJ, Makarewicz AJ, Bright IJ, Lucero MY, Hiddessen AL, Legler TC, Kitano TK, Hodel MR, Petersen JF, Wyatt PW, Steenblock ER, Shah PH , Bousse LJ, Troup CB, Mellen JC, Wittmann DK, Erndt NG, Cauley TH, Koehler RT, So AP, Dube S, Rose KA, Montesclaros L, Wang S, Stumbo DP, Hodges SP, Romine S, Milanovich FP, White HE, Regan JF, Karlin-Neumann GA, Hindson CM, Saxonov S, Colston BW. High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. *Anal Chem*. 2011;83:8604–8610.
23. Suo T, Liu X, Feng J, Guo M, Hu3 W, Guo D, Ullah2 H, Yang Y, Zhang Q, Wang X, Sajid M, Huang Z, Deng L, Chen T, Liu F, Ke X, Liu Y, Zhang Q, Liu Y, Xiong Y, Chen1 G, Lan K, Chen Y. ddPCR: a more sensitive and accurate tool for SARS-CoV-2 detection in low viral load specimens. *medRxiv*. 2020. DOI: 10.1101/2020.02.29.20029439
24. Liu Y, Ning Z, Chen Y, Guo M, Liu Y, Gali NK, Sun L, Duan Y, Cai J, Westerdahl D, Liu X, Xu K, Ho K, Kan H, Fu Q, Lan K. Aerodynamic analysis of SARS-CoV-2 in two Wuhan hospitals. *Nature*. 2020;582:557–560.
25. Wang J, Cai K, Zhang R, He X, Shen X, Liu J, Xu J, Qiu F, Lei W, Wang J, Li X, Gao Y, Jiang Y, Xu W, Ma X. Novel One-Step Single-Tube Nested Quantitative Real-Time PCR Assay for Highly Sensitive Detection of SARS-CoV-2. *Anal Chem*. 2020;13:9399–9404.
26. Goode T, Ho WZ, Connor TO, Busteed S, Douglas SD, Shanahan F, Connell JO. Nested RT-PCR. In: O'Connell J. RT-PCR Protocols. *Method Mol Biol*. 2002;193;65-79. Humana Press. https://doi.org/10.1385/1-59259-283-X:065

27. Jiang SS, Chen TC, Yang JY, Hsiung CA, Su IJ, Liu YL, Chen PC, Juang JL. Sensitive and quantitative detection of severe acute respiratory syndrome coronavirus infection by real-time nested polymerase chain reaction. *Clinic Infect Dis.* 2004;38:293-296.

28. Peiris JSM, Chu CM, Cheng VCC, Chan KS, Hung IFN, Poon LLM, Law KI, Tang BSF, Hon TYW, Chan CS, Chan KH, Ng JSC, Zheng BJ, Ng WL, Lai RWM, Guan Y, Yuen KY, HKU/UCH SARS Group. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. *Lancet.* 2003;361:1767-1772.

29. Nagamine K, Hase T, Notomi T. Accelerated reaction by loopmediated isothermal amplification using loop primers. *Mol Cell Probes*. 2002;16:223–9.

30. Green K, Winter A, Dickinson R, Graziadio S,Wolff R, Mallett S, Allen1 AJ. What tests could potentially be used for the screening, diagnosis and monitoring of COVID-19 and what are their advantages and disadvantages?. Centre for Evidence-Based Medicine, Oxford. 2020. Available at: https://www.cebm.net/covid-19/what-tests-could-potentially-be-used-for-the-screening-diagnosis-and-monitoring-of-covid-19-and-what-are-their-advantages-and-disadvantages/

31. James AS, Al-alwneh JI. COVID-19 infection diagnosis: potential impact of isothermal amplification technology to reduce community transmission of SARS-COV-2. *Diagnostics*. 2020;10(6):399.

32. Gallas-Lindemann C, Sureshkumar P, Noack MJ, Sotiriadou I. Loop-Mediated Isothermal Amplification: An Advanced Method for the Detection of Giardia. In: Rodriguez-Morales AJ. Current Topics in Giardiasis, IntechOpen. 2017;109-133.

33. Lu R, Wu X, Wan Z, Li Y, Jin X, Zhang C. A Novel Reverse Transcription Loop-Mediated Isothermal Amplification Method for Rapid Detection of SARS-CoV-2. *Int J Mol Sci.* 2020;21:2826.

34. Song J, Liu C, Mauk MG, Rankin SC, Lok JB, Greenberg RM, Bau HM. Two-StageIsothermal Enzymatic Amplification for Concurrent Multiplex Molecular Detection. *Clin Chem*. 2017;63(3):714–22.

35. El-Tholoth M, Bau HH, Song J. A Single and Two-Stage, Closed-Tube, Molecular Test for the 2019 Novel Coronavirus (COVID-19) at Home, Clinic, and Points of Entry. *ChemRxiv*. 2020. https://doi.org/10.26434/chemrxiv.11860137.v1

36. Kashir J, Yaqinuddin A. Loop mediated isothermal amplification (LAMP) assays as a rapid diagnostic for COVID-19, *Med Hypoth.* 2020;141:109786. doi: https://doi.org/10.1016/j.mehy.2020.109786

37. Behrmann O, Bachmann I, Spiegel M, Schramm M, Wahed, AAI, Dobler G. Dame G, Hufert FT. Rapid detection of SARS-CoV-2 by low volume real-time single tube reverse transcription recombinase polymerase amplification using an exo probe with an internally linked quencher (exo-IQ). *Clin Chem*. 2020;66(8):1047-1054.

38. Jinek M, Jiang F, Taylor DW, Sternberg SH, Kaya E, Ma E, Anders C, Hauer M, Zhou K, Lin S, Kaplan M, Iavarone AT, Charpentier E, Nogales E, Doudna JA. Structures of Cas9 endonucleases reveal RNA-mediated conformational activation. *Science.* 2014;343:1247997.

39. Carter LJ, Garner LV, Smoot JW, Li Y, Zhou Q, Saveson CJ, Sasso JM, Gregg AC, Soares DJ, Beskid TR, Jervey SR, Liu C. Assay Techniques and Test Development for COVID-19 Diagnosis. *ACS Cent Sci*. 2020;6:591−605.

40. Zhang F, Abudayyeh OO, Gootenberg JS. A Protocol for Detection of COVID-19 Using CRISPR Diagnostics; Broad Institute, MIT: Cambridge, MA. 2020;1-8. Available at: https://www.broadinstitute.org/files/publications/special/COVID-19%20detection%20(updated).pdf

41. Hou, T, Zeng W, Yang M, Chen W, Ren L, Ai J, Wu J, Liao Y, Gou X, Li Y, Wang X, Su H, Gu B, Wang J, Xu T. Development and Evaluation of A CRISPR-Based Diagnostic For 2019-Novel Coronavirus. *medRxiv*. 2020. DOI: https://doi.org/10.1101/2020.02.22.20025460

42. O'Connell MR. Molecular Mechanisms of RNA Targeting by Cas13-Containing Type VI CRISPR-Cas Systems. *J Mol Biol.* 2019;431: 66−87.

43. Broughton JP, Deng X, Yu G, Fasching CL, Servellita V, Singh J, Miao X, Streithorst JA, Granados A, Gonzalez AS, Zorn K, Gopez A, Hsu E, Gu W, Miller S, Pan CY, Guevara H, Wadford DA, Chen JS, Chiu CY. CRISPR–Cas12-based detection of SARS-CoV-2. *Nat Biotechnol*. 2020;38**,**870–874.

44. Joung J, Ladha A, Saito M, Segel M, Bruneau R, Huang MW, Kim NG, Yu X, Li J, Walker BD, Greninger AL, Jerome KR, Gootenberg JS, Abudayyeh OO, Zhang F. Point-of-care testing for COVID-19 using SHERLOCK diagnostics. *MedRxiv*. 2020;2020.05.04.20091231. doi: https://doi.org/10.1101/2020.05.04.20091231

45. Chen Q, Li J, Deng Z, Xiong W, Wang Q, Hu YQ. Comprehensive detection and identification of seven animal coronaviruses and human respiratory coronavirus 229E with a microarray hybridization assay. *Intervirol.* 2010;53(2): 95−104.

46. Guo X, Geng P, Wang Q, Cao B, Liu B. Development of a single nucleotide polymorphism DNA microarray for the detection and genotyping of the SARS coronavirus. *J Microbiol Biotechnol*. 2014;24 (10):1445−1454.

47. Li C, Zhao C, Bao J, Tang B, Wang Y, Gu B. Laboratory Diagnosis of Coronavirus Disease-2019 (COVID-19). *Clinic Chim Acta*. 2020;510: 35-46.

48. Kumar R, Nagpal S, Kaushik S, Mendiratta S. COVID-19 diagnostic approaches: different roads to the same destination. *Virus Dis*. 2020;31(2):97–105.

49. Sebastien M, Chiumenti M, Jonghe KD, Glover R, Haegeman A, Koloniuk I, Komınek P, Kreuze J, Kutnjak D, Lotos L, Maclot F, Maliogka V, Maree HJ, Olivier T, Olmos A, Pooggin MM, Reynard JS, Ruiz-Garcıa AB, Safarova D, Schneeberger PHH, Sela N, Turco S, Vainio EJ, Varallyay E, Verdin E, Westenberg M, Brostaux Y, Candresse T. Virus detection by high-throughput sequencing of small RNAs: large scale performance testing of sequence analysis strategies strategies. *Phytopathol*. 2019;109(3):488–97.

50. Meyer B, Drosten C, Müller MA. Serological assays for emerging coronaviruses: challenges and pitfalls. *Virus Res*. 2014;194:175-83.

51. Bai SL, Wang JY, Zhou YQ, Yu DS, Gao XM, Li LL, Yang F. Analysis of the first cluster of cases in a family of novel coronavirus pneumonia in Gansu Province. *Zhonghua yu fang yi xue za zhi*. *Chinese J Prev med*. 2020;54(0):E005.

52. Xiao SY, Wu Y, Li J. Evolving status of the 2019 novel coronavirus infections: proposal of conventional serologic assays for disease diagnostics and infection monitoring. *J Med Virol.* 2020;92:-464-467.

53. Justiz Vaillant AA, Ramphul K. Immunoglobulin. In: StatPearls. Treasure Island, FL: StatPearls Publishing; 2020. Available at: http://www.ncbi.nlm.nih.gov/books/NBK513460/.

54. Gronvall G, Connell N, Kobokovich A, West R, Warmbrod KL, Shearer MP, Mullen L, Inglesby T. Developing a National Strategyfor Serology (Antibody Testing) in the United States. Johns Hopkins University. 2020. Available at: https://www.centerforhealthsecurity.org/our-work/pubs_archive/pubs-pdfs/2020/200422-national-strategy-serology.pdf

55. Okba NMA, Müller MA, Li W, Wang C, GeurtsvanKessel CH, Corman VM, Lamers MM, Sikkema RS, de Bruin E, Chandler FD, Yazdanpanah Y, Hingrat QL, Descamps D, Houhou-Fidouh N, Reusken CBEM, Bosch BJ, Drosten C, Koopmans MPG, Haagmans BL. Severe acute respiratory syndrome coronavirus 2-specific antibody responses in coronavirus disease 2019 patients. *Emerg Infect Dis*. 2020;(7):1478-1488.

56. Tan W, Lu Y, Zhang J, Wang J, Dan Y, Tan Z, He X, Qian C, Sun Q, Hu Q, Liu H, Ye S, Xiang X, Zhou Y, Zhang W, Guo Y, Wang XH, He W, Wan X, Sun F, Wei Q, Chen C, Pan G, Xia J, Mao Q, Chen Y, Deng G. Viral kinetics and antibody responses in patients with COVID-19. *medRxiv*. 2020. doi:10.1101/2020.03.24.20042382

57. To KK, Tsang OT, Leung WS, Tam AR, Wu TC, Lung DC, Yip CCY, Cai JP, Chan JMC, Chik TSH, Lau DPL, Choi CYC, Chen LL, Chan WM, Chan KH, Ip JD, Ng ACK, Poon RWS, Luo CT, Cheng VCC, Chan JFW, Hung IFN, Chen Z, Chen H, Yuen KY. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *Lancet Infect Dis*. 2020;20(5):565-574.

58. Wu LP, Wang NC, Chang YH, Tian XY, Na DY, Zhang LY, Zheng L, Lan T, Wang LF, Liang GD. Duration of antibody responses after severe acute respiratory syndrome. *Emerg infect dis.* 2007;13(10): 1562–1564. https://doi.org/10.3201/eid1310.070576

59. Li X, Geng M, Peng Y, Meng L, Lu S. Molecular immune pathogenesis and diagnosis of COVID-19. *J Pharm Anal.* 2020;10:102-108.

60. Xie L, Sun C, Luo C, Zhang Y, Zhang J, Yang J, Zhang J, Yang J, Li J. SARS-CoV-2 and SARS-CoV Spike-RBD structure and receptor binding comparison and potential implications on neutralizing antibody and vaccine development. *bioRxiv*. 2020; 1-18. doi: https://doi.org/10.1101/2020.02.16.951723

61. World Health Organization report. Advice on the use of point-of-care immunodiagnostic tests for COVID-19. 2020b. Available at: https://www.who.int/news-room/commentaries/detail/advice-on-the-use-of-point-of-care-immunodiagnostic-tests-for-covid-19

62. Bruning AHL, Leeflang MMG, Vos JMBW, Spijker R, de Jong MD, Wolthers KC, Pajkrt D. Rapid Tests for Influenza, Respiratory Syncytial Virus, and Other Respiratory Viruses: A Systematic Review and Meta-analysis. *Clin Infect Dis.* 2017;65(6):1026–32.

63. U.S. Food & Drug Administration (FDA). Sofia 2 SARS Antigen FIA. 2020. Available at: https://www.fda.gov/media/137886/download

64. Zhang W, Du RH, Li B, Zheng XS, Yang XL, Hu B, Wang YY, Xiao GF, Yan B, Shi ZL, Zhou P. Molecular and Serological Investigation of 2019-nCoV Infected Patients: Implication of Multiple Shedding Routes. *Emerg Microb Infect*. 2020;9(1):386−389.

65. Otto MA FDA grants emergency authorization for first rapid antibody test for covid-19. 2020. Available at: https://www.the-hospitalist.org/hospitalist/article/220225/coronavirus-updates/fda-grants-emergency-authorization-first-rapid

66. Whiting P, Singatullina N, Rosser JH. Computed Tomography of the Chest: I. Basic Principles. *BJA Educat.* 2015;15(6):299–304.

67. Bernheim A, Mei X, Huang M, Yang Y, Fayad ZA, Zhang N, Diao K, Lin B, Zhu X, Li K, Li S, Shan H, Jacobi A, Chung M. Chest CT Findings in Coronavirus Disease-19 (COVID-19): Relationship to Duration of Infection. *Radiol*. 2020; 295:685–691.

68. Kobayashi Y, Mitsudomi T. Management of Ground-Glass Opacities: Should All Pulmonary Lesions with Ground-Glass Opacity Be Surgically Resected?. Transl. *Lung Cancer Res*. 2013;2(5):354–363.

69. Pan F, Ye T, Sun P, Gui S, Liang B, Li L, Zheng D, Wang J, Hesketh RL, Yang L, Zheng C. Time Course of Lung Changes On Chest CT During Recovery From 2019 Novel Coronavirus (COVID-19) Pneumonia. *Radiol.* 2020;295:715-721.

70. Long C, Xu H, Shend Q, Zhang X, Fana B, Wanga C, Zenga B, Lia Z, Lia X, Lia H. Diagnosis of the Coronavirus disease (COVID-19): rRT-PCR or CT? *Europ J Radiol*. 2020;126:108961.

71. Lee EYP, Ng MY, Khong PL. COVID-19 pneumonia: what has CT taught us? *Lancet Infect Dis.* 2020;4: 384–385.

72. Fang Y, Zhang H, Xie J, Lin M, Ying L, Pang P, Ji W. Sensitivity of Chest CT for COVID-19: Comparison to RT-PCR. *Radiol.* 2020;296(2):E115-E117.

73. Xie X, Zhong Z, Zhao W, Zheng C, Wang F, Liu J. Chest CT for Typical 2019-nCoV Pneumonia: Relationship to Negative RT-PCR Testing. *Radiol.* 2020; 296(2):E41-E45.

74. Ai T, Yang Z, Hou H, Zhan C, Chen C, Lv W, Tao Q, Sun Z, Xia L. Correlation of Chest CT and RT-PCR Testing in Coronavirus Disease 2019 (COVID-19) in China: A Report of 1014 Cases. *Radiol*. 2020; 296(2):E32-E40.

75. Seo G, Lee G, Kim MJ, Baek S-H, Choi M, Ku KB, Lee C-S, Jun S, Park D, Kim HG, Kim S-J, Lee J-O, Kim BT, Park EC, Kim SI. Rapid Detection of COVID-19 Causative Virus (SARS-CoV-2) in Human Nasopharyngeal Swab Specimens Using Field-Effect Transistor-Based Biosensor. *ACS Nano*. 2020;14: 5135−5142.

76. Mokhtarzadeh A, Eivazzadeh-Keihan R, Pashazadeh P, Hejazi M, Gharaatifar N, Hasanzadeh M, Baradaran B, de la Guardia M. Nanomaterial-based biosensors for detection of pathogenic virus. *Trends Anal Chem*. 2017;97:445-457.

77. Arugula MA, Simonian A. Novel trends in affinity biosensors: current challenges and perspectives. *Meas Sci Technol.* 2014;25(3): 032001.

78. Bagalkot V, Zhang L, Levy-Nissenbaum E, Jon S, Kantoff PW, Langer R, Farokhzad OC. Quantum dot aptamer conjugates for synchronous cancer imaging, therapy, and sensing of drug delivery based on bi-fluorescence resonance energy transfer. *Nano Lett*. 2007;7:3065–70.

79. Cheng MP, Papenburg J, Desjardins, M; Kanjilal S, Quach C, Libman M, Dittrich S, Yansouni CP. Diagnostic Testing for Severe Acute Respiratory Syndrome–Related Coronavirus-2. *Annal Inter Med*. 2020;172:726-734.

80. Chen L, Liu HG, Liu W, Liu J, Liu K, Shang J, Deng Y, Wei S. Analysis of clinical features of 29 patients with 2019 novel coronavirus pneumonia. Zhonghua Jie He He Hu Xi Za Zhi. *Chinese J tubercul respirat dis.* 2020;43:203-208.

81. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Xia J, Yu T, Zhang X, Zhang L. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet.* 2020;395:507-513.

82. Huang C, Wang Y, Li X, ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* 2020;395:497-506.

83. Moitra P, Alafeef M, Dighe K, Frieman MB, Pan D. Selective Naked-Eye Detection of SARS-CoV-2 Mediated by N Gene Targeted Antisense Oligonucleotide Capped Plasmonic Nanoparticles. *ACS Nano*. 2020;14(6):7617-7627.

84. Zhao Z, Cui H, Song W, Ru X, Zhou W, Yu X. A simple magnetic nanoparticles-based viral RNA extraction method for efficient detection of SARS-CoV-2. bioRxiv. 2020. doi: https://doi.org/10.1101/2020.02.22.961268

85. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF, Tan W. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med*. 2020;382(8):727-733.

86. Hopman J, Allegranzi B, Mehtar S. Managing COVID-19 in Low- and Middle-Income Countries. *JAMA*. 2020;323(16):1549-1550.

87. Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, Hase T. Loop-Mediated Isothermal Amplification of DNA. *Nucleic Acids Res*. 2000;28(12):E63.

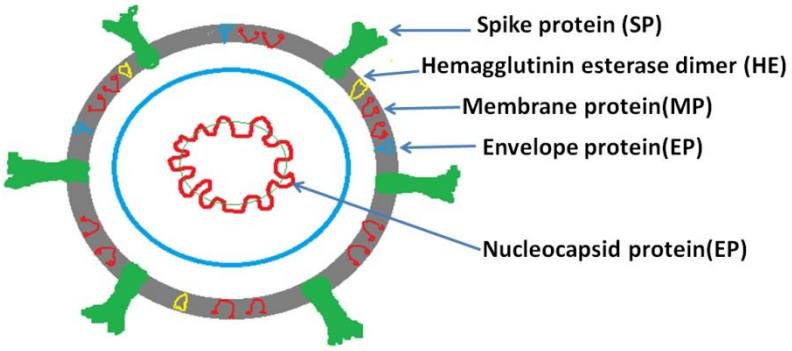

Figure 1. Schematic diagram of SARS-CoV-2

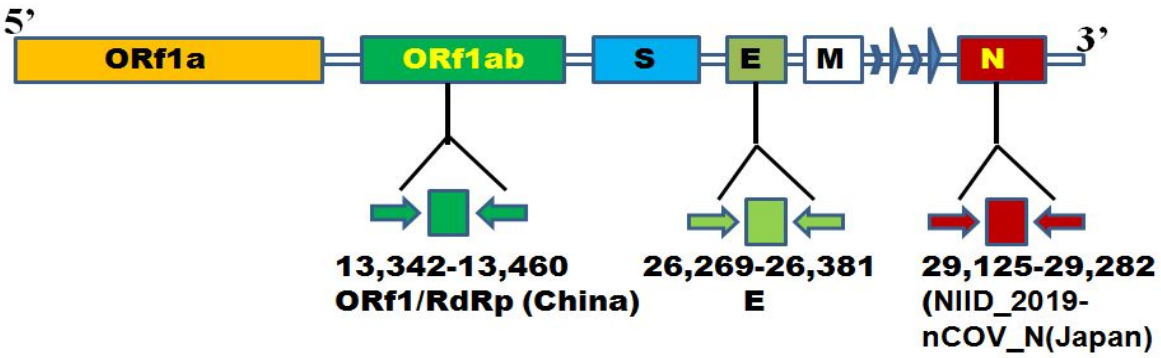

Figure 2. Relative positions of qRT-PCR primer-probe set on the SARS-CoV-2 (Slight modification to Jung et al.[12]), Numbers below amplicon are genome positions. Orf1: open reading frame 1; RdRp: RNA-dependent RNA polymerase gene; S: spike protein gene; E: envelop protein gene, N: nucleocapsid protein gene.

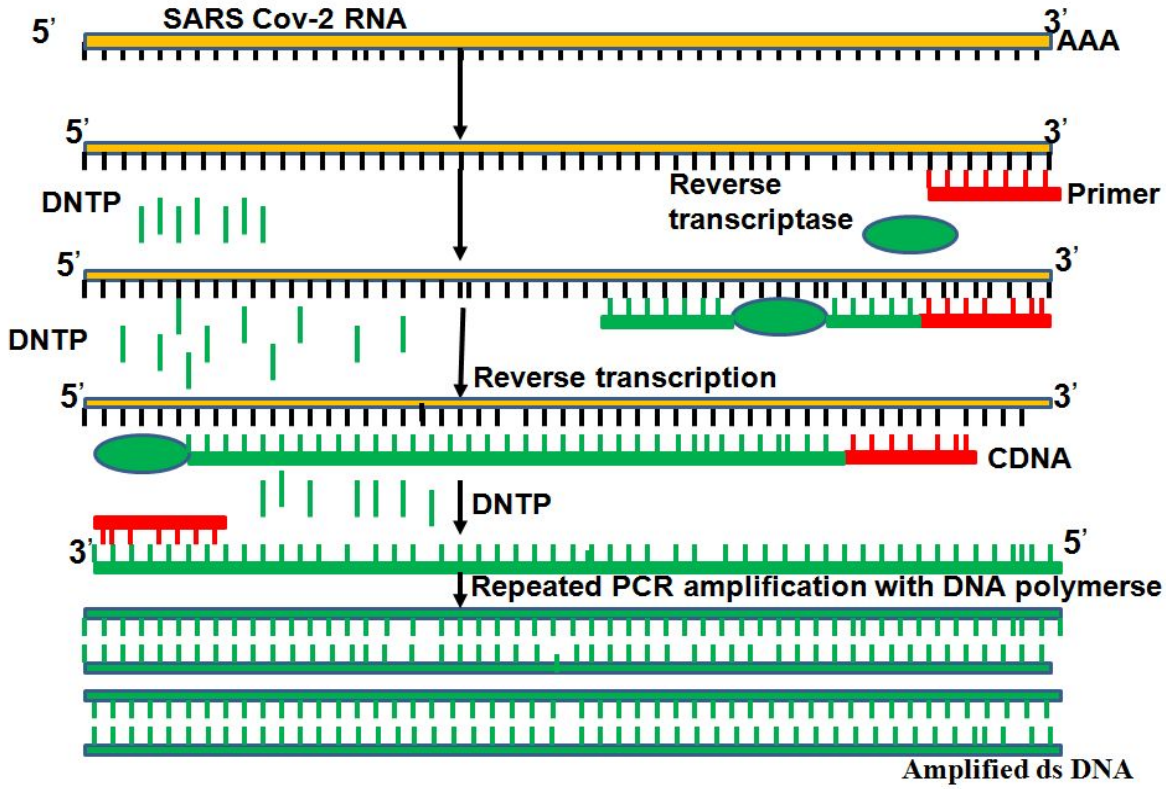

Figure 3. Schematic representation of RT-PCR for amplification of SARS CoV-2 ds DNA

2.2 Droplet digital PCR (ddPCR)

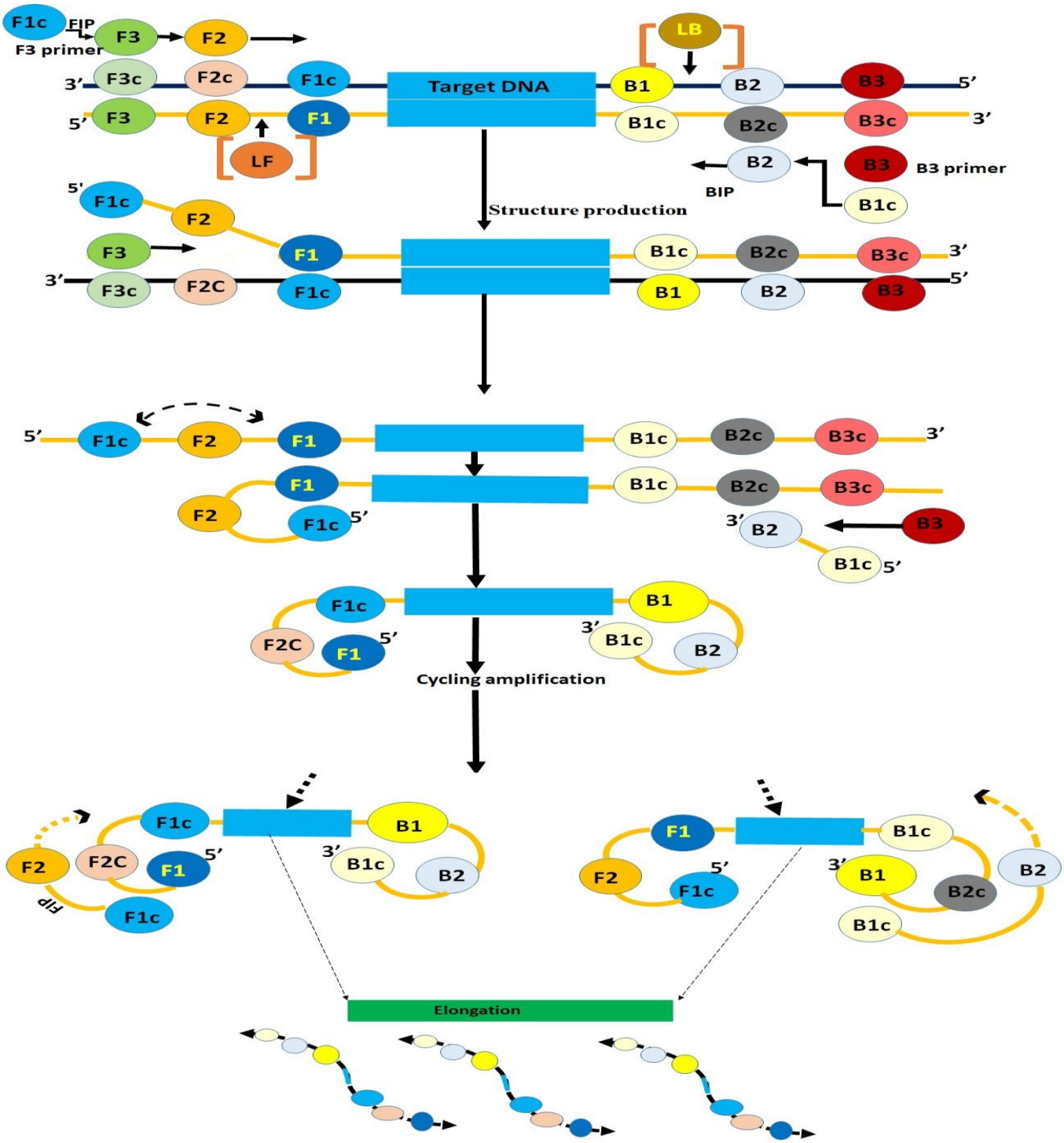

Figure 4. Loop-Mediated Isothermal Amplification

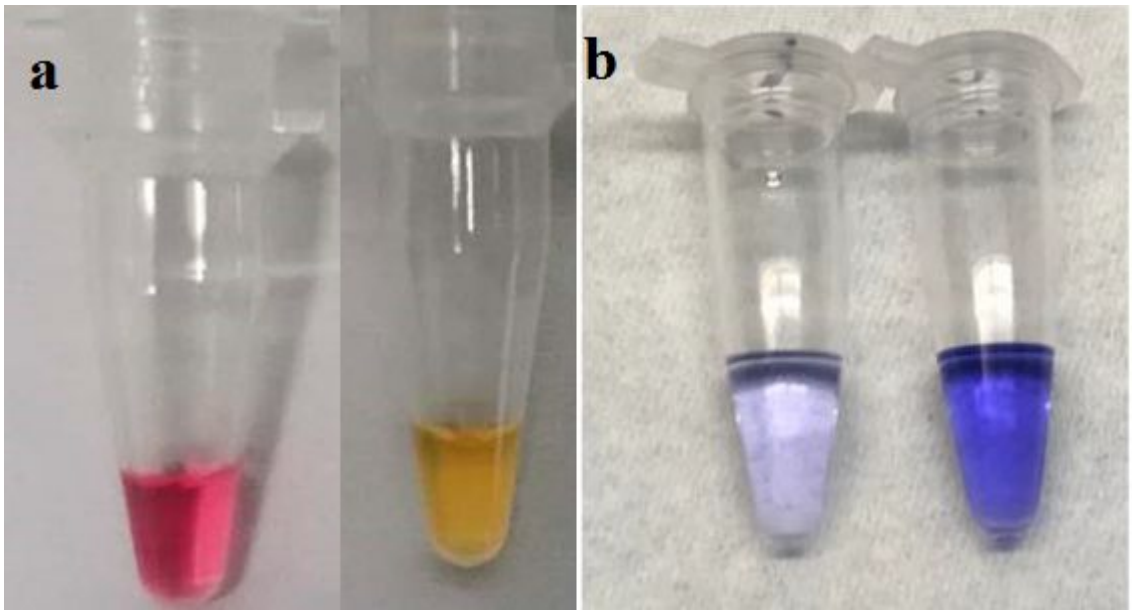

Figure 5. Visual detection of SARS-CoV-2 in RT-LAMP diagnostic method via colorimetric change from (a) from red (negative) to yellow (positive) (Lu et al. 2020). (b) from lighter (negative) to dark (positive) [35]. (Sorce: Kashir and Yaqinuddin [36]) (Free Copyright permissions granted for by Elsevier Ltd. for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source as long as the COVID-19 resource centre active)

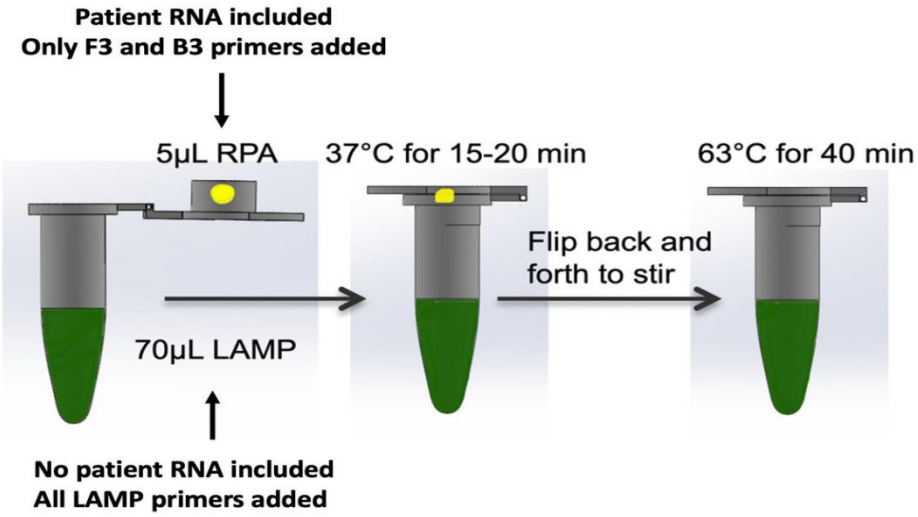

Figure 6. Schematic representation of Penn-RAMP procedure [35]. (Sorce: Kashir and Yaqinuddin [36]) (Free Copyright permissions granted for by Elsevier Ltd. for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source as long as the COVID-19 resource centre active)

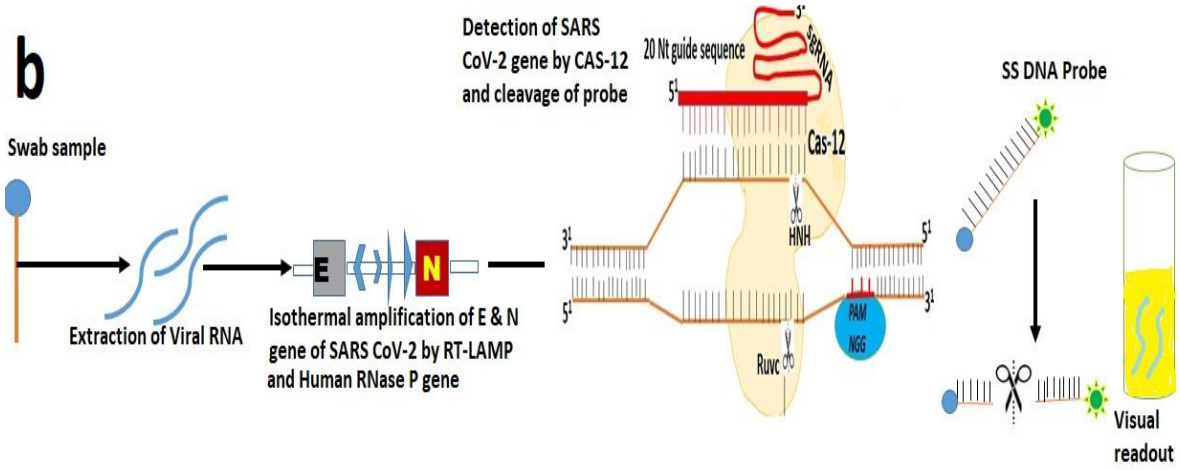

Figure 7. Schematic representation of (a) nuclease activity of Cas1 3a, activated upon specific binding of gRNA to the *Orf1ab* gene. Fluorescent signal produced from cleaved probes is captured and indicates the presence of 2019-nCoV (With prior copyright permission Hou, et al. [41]). (b) Schematic of SARS-CoV-2 DETECTOR workflow. Conventional RNA extraction can be used as an input to DETECTOR (LAMP preamplification and Cas12-based detection for E gene, N gene and RNase P), which is visualized by a fluorescent reader (Slight modification to Broughton et al. [43]).

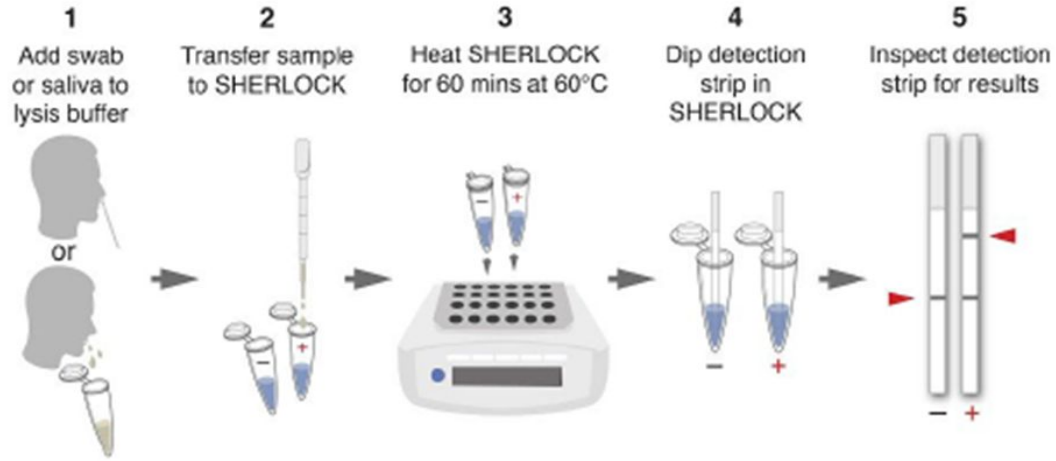

Figure 8. Steps of the one-step STOP Covid test (With prior copyright permission from Joung et al. [44])

2.6. Microarray.

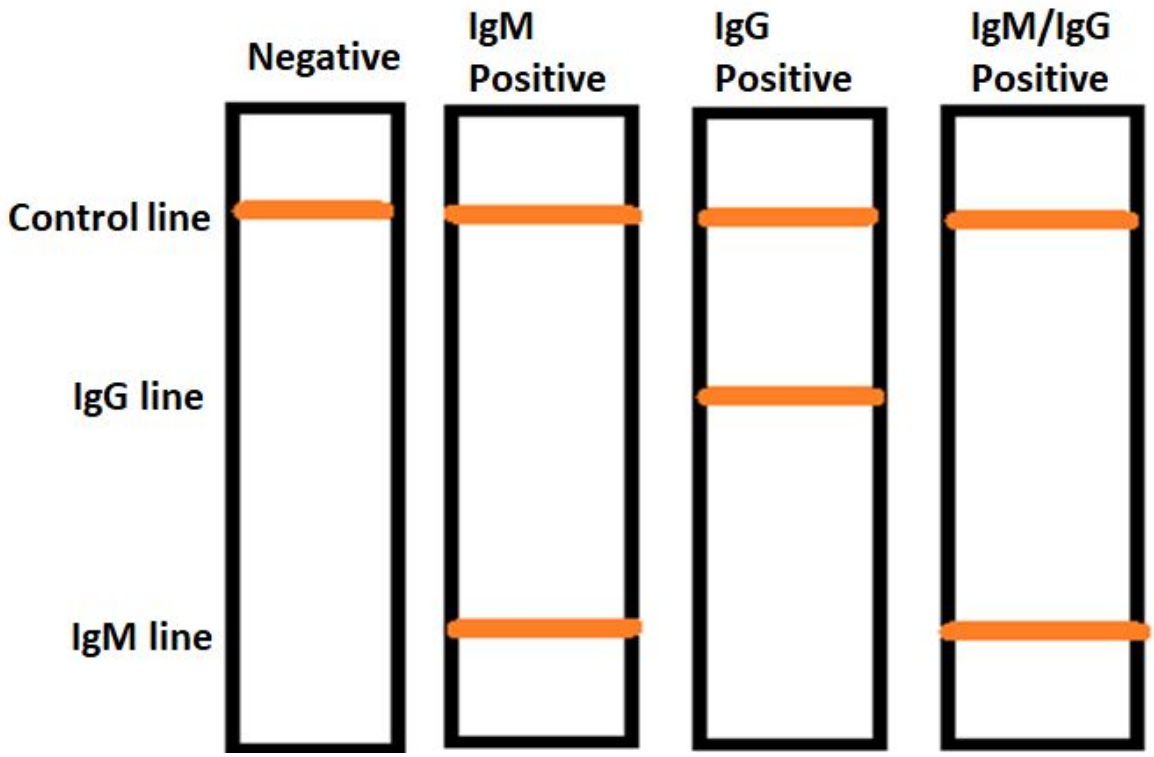

Figure 9. Validity test of RDT technique [30]

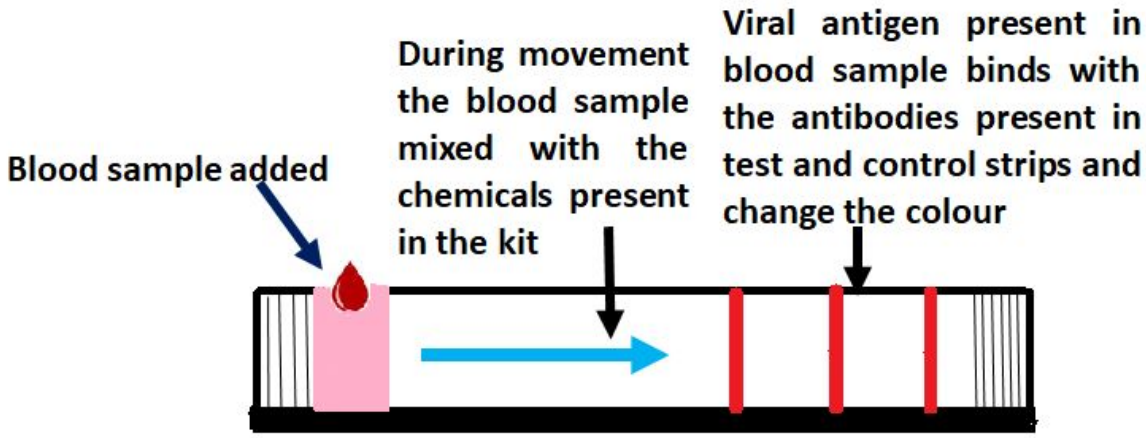

Figure 10. Detection of antibodies by RDT method [30]

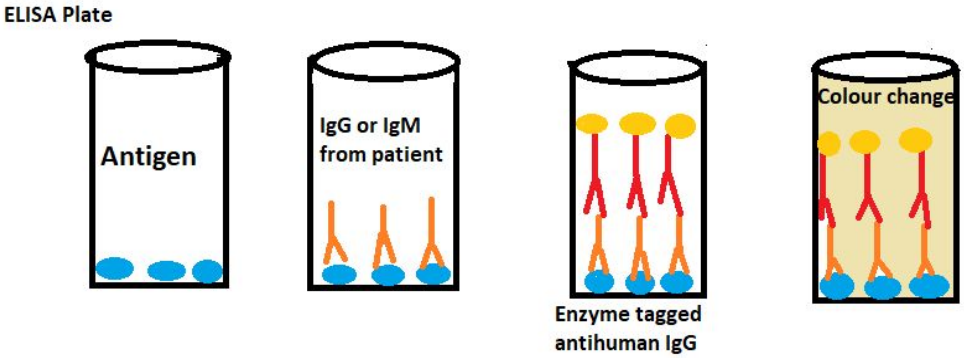

Figure 11. Enzyme-linked immunosorbent assay for COVID-19 antibody detection [30].

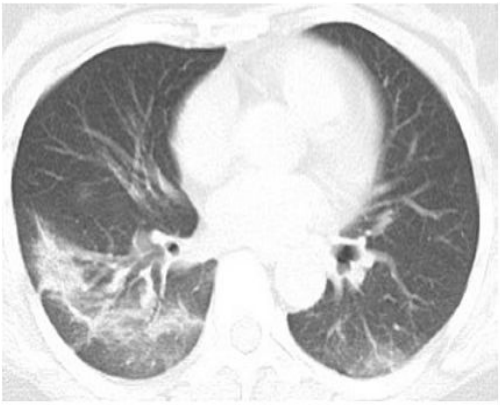

Figure 12. CT scan images showing patchy consolidations and ground glass opacities in both lungs of a covid-19 patient [70] (With prior copy right permission from the Journal Manager of European Journal of Radiology).

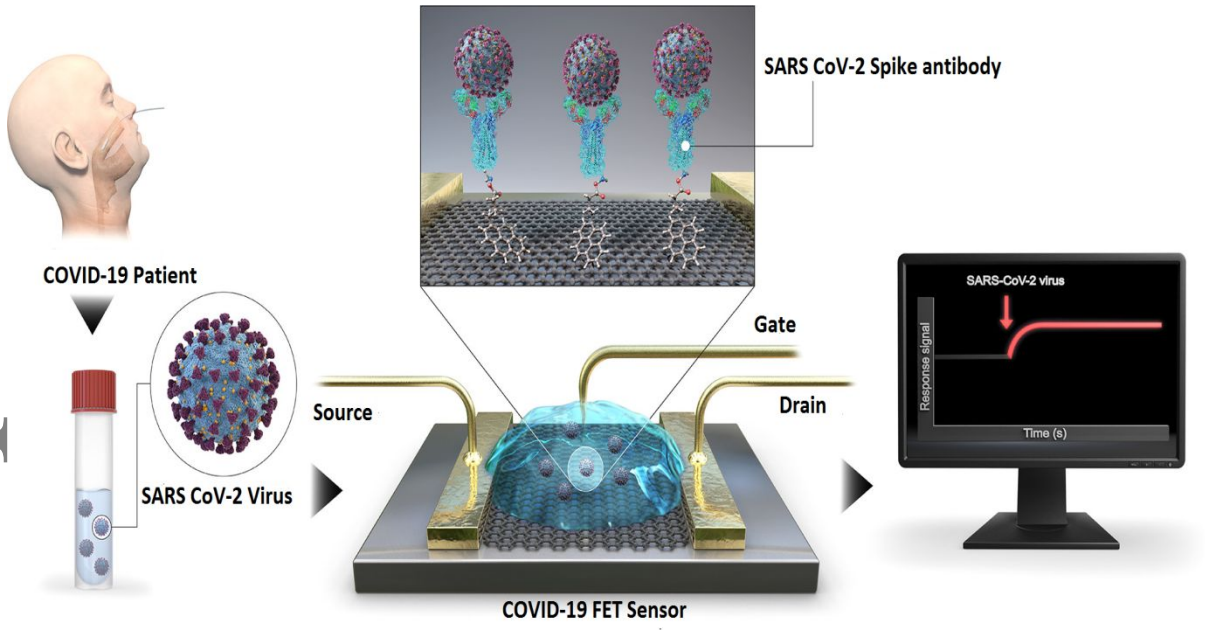

Figure 13. Field-effect transistor (FET)-based biosensing device for detection of SARS-CoV-2 (Seo et al.[76]). (Sorce: https://pubs.acs.org/doi/10.1021/acsnano.0c02823), (further permission related to the material excerpted should be directed to the ACS).

Table 1 Different diagnostic techniques for detection of COVID-19.

Diagnostic Technology	Detection	Type of sample	Laboratory/ point of care test	Advantage	Disadvantage	Reference
RT-PCR	Viral RNA	Nasopharyngeal swab, sputum, stool	Laboratory based	Specific detection & time saving	False negative results also detected in low viral load	[5, 12-16]
ddPCR	Viral RNA	Nasopharyngeal swab, sputum, stool	Laboratory based	It can detect SARS-Cov-2 accurately in low viral load samples and minimise false negative results.	More expensive and susceptible to exogenous contamination.	[23, 24]
Nested RT-PCR	Viral RNA	Nasopharyngeal swab,	Laboratory based	It can detect SARS-Cov-2 accurately in low viral load samples and minimise false negative results.	The lid opening after the first round of Nested PCR increases the risk of cross-contamination which may lead to false positive results	[25]
RT-LAMP	Viral RNA	Nasopharyngeal swab, sputum, stool	Laboratory based/ point of Care	Sensitive and specific results in less time, result visualize in eye, does not need a thermo cycler	Cumbersome to optimize primers and reaction conditions	[88]
Penn RAMP technology	Viral RNA	Nasopharyngeal swab	Laboratory based/ point of Care	Sensitivity of one-tube Penn-RAMP is 10 times higher than LAMP and RT-PCR.	Costly reagents such as polymerase and primers required more number than the LAMP	[35]

CRISPR	Viral RNA	Nasopharyngeal swab, bronchoalveolar lavage fluid	Laboratory based	Easy to perform and low cost In STOP COVID test even RNA extraction are not required	Sometimes gives false result	[39]
Microarray	Viral RNA	Nasopharyngeal swab, bronchoalveolar lavage fluid	Laboratory based	It can identify any mutations associated with SARS-CoV and can detect SNP associated with spike (S) gene of SARS-CoV-2	High cost of a single experiment, the large number of probe designs based on sequences of low-specificity, as well as the lack of control over the pool of analyzed transcripts.	[46]
Viral Sequencing	Viral RNA	Nasopharyngeal swab,	Laboratory based	Convenient, high sensitivity, suitable for detect samples with low viral load	Sophisticated instruments, Increase cost, requires trained person	[48]
Serology	Antibody/ Antigen	Blood	Laboratory based/ Point of Care	less complex than molecular tests	Antibody detection tests targeting COVID-19 may also cross-react with other pathogens and also false negative result may come at low antigen level at early stage of infection	[61]
ELISA	Antibody	Blood	Laboratory based	simple and cheap laboratory technique, important for vaccine development and convalescent plasma therapy	ELISA tests are not yet well established for SARS-CoV-2 COVID-19 testing,	[64]

CT Scan	Lungs	Images of chest	Point of care	CT scans have a higher sensitivity (86−98%) and improved false negative rates compared to RT-PCR.	Specificity is low (25%) because the imaging features overlap with other viral pneumonia	[70]
Biosensor	S protein of SARS-CoV-2	Nasopharyngeal swab, sputum, stool	Point of Care	Quick detection without any pre-treatment of the sample.	Cost effective	[76]
Biomarker	C-reactive protein, lymphocytes, and SARS-CoV-2 RNA	Nasopharyngeal swab and Blood	Laboratory based	Easy non-microbiologic rapid tests	Same Biomarkers are also abnormal in other illnesses and not specific enough to establish a diagnosis of COVID-19	[80]
Nanotechnology	SARS-CoV-2 RNA	Nasopharyngeal swab	Point of Care	High sensitivity, adopted in fully-automated RNA extraction systems, excellent RNA binding performances	Complex pre-treatment steps, requires skilful, expensive than qRT-PCR, with the risk of photobleaching	[84]
Viral culture	In vitro live virus	Human epithelial cell lines	Laboratory based	Important for identification, detection of mutation and development of vaccine	Low sensitivity as isolation is not 100%	[86]