COVID-19 detection: Comparison and accuracy of several diagnostic tests

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Abstract

The emergence of coronavirus disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus (SARS-CoV-2) has created a global alarming situation. Initially, diagnosis of the virus was conducted by a combination of different assays including; electron microscopy (EM), computed tomography (CT) scan and whole-genome sequencing. However, such time-consuming diagnosis has critically highlighted the need for a fast and specific testing approach for earlier detection. It is expected that the ongoing research advances and the continuing efforts for the fight against COVID-19 will soon bring a fruitful discovery in in vitro diagnostics techniques. Meanwhile, the efficiency and performance of any novel diagnostic approach should be censoriously analyzed before it comes into practice. The aims of this study were to evaluate and compare the different diagnostic tests recommended by the World Health Organization (WHO) for earlier detection of the novel SARS-CoV-2. We found that the Clustered regularly interspaced short palindromic sequence (CRISPR/Cas) detection has relatively higher efficiency, compared to all the other tests. Therefore from this report, we concluded that the discovery of CRISPR/Cas based in vitro diagnostics will minimize the time limit as well as the cost for COVID-19 detection.

Keywords: SARS-CoV-2, Pandemic, In vitro diagnostics, COVID-19, CRISPR/Cas

1. Introduction

The coronavirus disease 19 (COVID-19) has emerged at the end of December, 2019 in Wuhan city, China, which has created a panic situation all over the world (Lin et al., 2020; Wu et al., 2020). In the initial stages of the outbreak its impact was greatly on China, as Wuhan province was the epicenter, subsequently it spread to the other provinces of China, as reported by Lin et al., (2020); Wang et al., (2020a). Furthermore, Cucinotta and Vanelli, (2020); WHO, (2020a) added that since then the COVID-19 was spreading at an alarming rate, and effected many continents that led the situation to a worldwide pandemic, which has created a new challenge for human beings. The WHO reported that, since the 10th of May, 2020, the infection
caused by SARS-CoV-2 has spread in about 215 countries and territories, affecting nearly four million peoples with more than 265,862 confirmed deaths. The highest number of positive cases (≥ 10^6), have been reported in the United States of America (USA), followed by Spain, Italy and the United Kingdom (UK). Correspondingly, the highest numbers of death reports (≥ 69 ×10^3) have been received from the USA with an accelerated number of deaths in Italy, Spain, and France (WHO, 2020b). It is to be mentioned that the number of new cases in the Wuhan epicenter was greatly reduced at the end of March, while South Korea also have successfully controlled the spread of COVID-19 and succeeded in stopping the epidemic by taking several essential measures.

2. Structure of COVID-19

Recent studies of Gorbalenya et al., (2020); Pal et al., (2020) reported that the novel coronavirus (SARS-CoV-2), which causes severe acute respiratory syndrome have a crown-like structure, and it belong to a family of coronaviruses known as Coronaviridae, which comes in the order of Nidovirales. Wrapp et al., (2020) reported that the spike proteins are responsible for the attachment of the virus to the host cell receptors causing pneumonia, and may lead to other respiratory illness with symptom ranging from mild to severe state. Cui et al., (2019); Andersen et al., (2020) added that the genome sequence of the SARS-CoV-2 show homology with the previously reported coronaviruses, which have been seen in 2002 as they were the sources of pneumonia infections in case of severe acute respiratory syndrome (SARS), and in 2012 during the middle east respiratory syndrome (MERS). Due to high homology with SARS-CoV, the virus is coined as coronavirus (2019-nCoV) after the genome sequencing (Zhang and Holmes, 2020). Several recent studies of Khan et al., (2020); Shereen et al., (2020); Wang et al., (2020a) revealed that the SARS-CoV-2 is a positive sense, single-stranded RNA virus with a genome of 26-32 kb, and size of around 60-200 nm in length. Its genetic characteristic features are determined by the open reading frame (ORF) genes, responsible for expression of envelope (E) proteins, spikes (S) proteins, nucleocapsid (N) proteins and membrane (M) proteins, as shown in Fig. (1).

Fig. 1. Structural features of SARS-CoV-2: The outer surface contains bounded envelope proteins (E) with protruding spike proteins (S), membrane proteins (M) are found embedded in the lipid bilayer of the SARS-CoV-2, while nucleocapsid proteins (N) are present inside along with single-strand RNA

According to Shereen et al., (2020); Sun et al., (2020); Wang et al., (2020b), the receptor-binding domain (RBD) of spike subunit 1 (S1) is known to be crucial for causing the viral infection as it recognizes and binds with the angiotensin-converting enzyme two receptors (ACE2), which is usually present in the host lower respiratory tract. Lu et al., (2020); Zhang and Holmes, (2020) are claiming that all types of human coronaviruses may share a common origin, the reservoir of which are considered to be the bats.

3. Symptoms of SARS-CoV-2 infection

Instead of the low fatality rate of SARS-CoV-2, it is highly contagious as compared to SARS and MERS, due its rapid transmission rate (Chan et al., 2020a; Liu et al., 2020a). Peoples infected with COVID-19 may express symptoms within 14 days’ time period after being exposed or come in close contact with an infected person and/or an object carrying the virus.
particle. Recent studies conducted by Chen et al., (2020a); Huang et al., (2020a); Singhal, (2020) revealed that symptoms of COVID-19 may be mild to severe depending on the host immune response and his/her age, they include fever, dry cough, body ache, muscle fatigue, runny nose and shortness of breath. Old age and immune-compromised peoples are highly prone to COVID-19 with a much higher fatality rate; however, it may be completely asymptomatic in some cases. In the young population, about 80% of them experience mild symptom only (Singhal, 2020; Udugama et al., 2020). Moreover, Chan et al., (2020a) added that in higher severe cases of the disease, it may also lead to diarrhea, acute respiratory distress syndrome (ARDS), and even to renal failure.

4. Treatment of COVID-19

There is no specific treatments or vaccines discovered for COVID-19; however, several studies conducted by Colson et al., (2020); Hu et al., (2020a) reported that symptomatic use of antibiotics and certain antiviral drugs along with a combination of chloroquine and hydroxychloroquine showed a little satisfactory results.

Accordingly, as there is a lack of in vivo studies for the current drugs related to COVID-19, experts recently reported the possible adverse effects of the anti-malarial drugs, thus there might be a potential threat to the underlying peoples having secondary disorders (Juurlink, 2020; Owens, 2020). For finding the effective drug, the latest study on cell lines with a pre-human trial, claimed the use of Atazanavir as an antiviral drug for inhibiting the SARS-CoV-2.

On the other hand, the Food and drug administration (FDA) approved the anti-parasitic drug named Ivermectin, which showed slight inhibitory effects in a lab trial, and was reported as being effective when its activity was checked in vivo on cell culture (Caly et al., 2020). Since at present there is no highly effective drug or vaccine available to combat the COVID-19, thus the primary tactic to fight the current pandemic is the early detection and diagnosis of infected peoples on time to isolate them, and avoid further spread of the disease. In the current study, we are going to discuss certain promising and rapid diagnostic tests that can be used for early detection of COVID-19.

5. Diagnostic tests for detection of SARS-CoV2

5.1. Real-time reverse transcription-polymerase chain reaction (RT-PCR)

The RT-PCR is one of the highly sensitive and specific methods used for COVID-19 detection (East et al., 2020; Feigelman et al., 2020). The genome sequences of SARS-CoV-2 revealed by the National Center for Biotechnology Information (NCBI) made detection of COVID-19 easier through RT-PCR assay (NCBI, 2020). The RT-PCR phenomenon for detection of COVID-19 is based on reverse transcription; for this reason, the RNA extracted from patient’s samples (may be lower or upper respiratory specimens such as nasopharyngeal or oropharyngeal swabs, sputum etc.) is initially reversed transcribed to complementary DNA (cDNA). After amplification, the RNA is finally detected through the real-time quantitative PCR, with primers and probes specifically designed for COVID-19 (Won et al., 2020). For this purpose, the particular primer sets and specific guideline for detection of COVID-19 through RT-PCR were made available by the Center for Disease Control (CDC) USA, according to CDC, (2020b).

The design of COVID-19-RT-PCR process may be one step, thus providing a rapid and reproducible result, or it may be normally performed in a two-step process which includes; 1) sequence alignment with designing primers and probes, and 2) assay optimization, as reported recently by Corman et al., (2020). Different institutes and departments have their own designed system of RT-PCR for COVID-19 detection, targeting either nucleocapsid gene (N), RNA dependent RNA polymerase/helicase (RdRp/ Hel) spike (S), or envelope gene (E) (Chan et al., 2020a; Udugama et al., 2020). The main steps involved in RT-PCR are presented in Fig. (2).
Fig. 2. RT-PCR steps for SARS-CoV-2 detection: Nasopharyngeal/oropharyngeal swab samples taken from patients are processed for RNA extraction. The extracted RNA is reverse transcribed to complementary DNA (cDNA) through reverse transcriptase enzyme. After that, the cDNA is amplified through polymerase enzyme forming multiple copies, which is finally detected as SARS-CoV2 nucleic acid.

Recently, CDC. (2020a); Chan, et al., (2020a) highlighted that for detection of SARS-CoV-2, the US Centers for Disease Control and Prevention uses a single RT-PCR assay, in which the extracted RNA is added to a master mixture. This master mixture primarily contains a DNA polymerase enzyme, a set of primers and a probe; containing a single oligonucleotide chain complementary to the targeted COVID-19 DNA, with a reporter and a quencher at each end. According to Wong and Medrano, (2005), during DNA amplification, the probe is cleaved and degraded on the base of 5’- 3’ exonuclease activity of the polymerase enzyme, which in turn will releases the reporter from the quencher thus permitting fluorescence.

This means that the intensity of fluorescence is highly proportional to the magnitude of the freshly synthesized DNA strands; on this basis the COVID-19 complementary DNA in any patient specimen can be quantified. Chan et al., (2020a) revealed that among all RT-PCR assays, the COVID-19-RdRp/ Hel (RNA dependent RNA polymerase/helicase) is considered to be highly sensitive and exceptionally specific for COVID-19 diagnosis, as there is minimum reported cross-reactivity with other human coronaviruses. Holshue et al., (2020) reported that in order to avoid any potential cross-reactivity and increase the assay performance, researchers in several countries have used more than one molecular target at a time, such as two targets loci from the nucleocapsid gene that were used in the US.

A recent study conducted by Won et al., (2020) reported that for early detection of SARS-CoV-2, an alternative low cost rapid diagnostic RT-PCR kit for COVID-19 using Trizol-based RNA purification and SYBR Green-based RT-PCR with a newly developed protocol has also been proposed, which can be practiced at any conventional Biosafety Level II laboratories (BSL II). Several studies have also revealed that the RNA-extraction step can be omitted
by the direct detection of SARS-CoV-2 through TaqMan™ 2019-nCoV Assay Kit v1 obtained from Thermo Fisher Scientific (Beltrán-Pavez et al., 2020). In spite of the high sensitivity and specificity of RT-PCR, certain inadequacies with this technique also do exist, such as the required PCR testing kits that are costly, thus reduced the availability of these kits in the community hospitals (Giri and Rana, 2020).

Moreover, Corman et al., (2020) added that the experimental inaccuracy or improper handling may sometimes lead to false positive or false negative results in initial PCR testing; accordingly such mini mismanagement may prolong the detection process. Moreover, rural areas have no possible PCR infrastructure to handle such heavy samples for COVID-19 screening.

5.2. Computed tomography (CT) scanning

A computed tomography scan acts as a supplementary analytic and diagnostic tool, which is highly effective when there is no available setting for PCR during emergency concerns. In addition, sometimes the RT-PCR fails to detect the gene responsible for COVID-19 due to low viral load, as it is observed in the case of patients being at the early stages of infection. Recently, Bai et al., (2020); Chan et al., (2020b); Huang et al., (2020b) reported that CT imaging enables the physician to detect COVID-19 patients with significant abnormalities in the lungs on the basis of certain ground-glass opacities.

Caruso et al., (2020); Hani et al., (2020); Zhang et al., (2020) added that although CT scan findings for COVID-19 depends on the disease severity, some common features of the chest CT which may help the radiologist in detecting COVID-19 include; multiple lobes involvement, bilateral and sub-pleural distribution, linear or peripheral consolidations, segmental vessel enlargement and showing a crazy-paving pattern. Studies of Caruso et al., (2020); Fang et al., (2020) revealed that although RT-PCR is a specific method which is used as a standard reference for COVID-19 detection; however, several studies reported that the sensitivity of CT scan in many cases was high as compared to PCR. Accordingly, during this COVID-19 pandemic, the CT scan played an important role in the early diagnosis of SARS-CoV-2 pneumonia. Recently, a deep learning-based model for COVID-19 detection through the high resolution computed tomography, showed 100% sensitivity with improved specificity (Chen et al., 2020b). Moreover, Long et al., (2020) added that severe cases of COVID-19 suspects with false-negative RT-PCR results can be diagnosed using the high-resolution CT scan.

Recent findings of Fan et al., (2020) study highlighted that apart from the positive role of CT scan, sometimes differential analysis between COVID-19 and other pulmonary syndromes/diseases is exceptionally difficult due to the overlap imaging features. However, several recent studies of Hu et al., (2020b); Mungmunpupintipantip and Wiwanitkit, (2020) demonstrated that certain asymptomatic cases of COVID-19 showed no any particular findings in the chest CT, so it can’t be trusted as a standard assay for COVID-19 diagnosis, but it will provide complementary information’s in severe cases only.

5.3. Immuno-assays/serological assays

The human adaptive immune system gets activated after entering of SAR-CoV-2 into the host cell, and then induces the production of various immune molecules, including cytokines TNF α and interleukins. Along with its different immunoglobulin proteins, the production of IgM and IgG also takes place. Haveri et al., (2020) demonstrated that IgM production starts within the first 3-4 days, whereas IgG antibodies are produced on the 6-8 days post-infection. The study of Xiang et al., (2020a) revealed that the detection of these antibodies may indicate the exposure of patients to SARS-CoV-2. According to Guo et al., (2020a), the existence of IgM antibody indicates a recent infection, while IgG indicates an earlier exposure to the virus, this could be determined on the basis of the highly specific antigen-antibody interaction. However, Zhao et al., (2020) highlighted that the current antibody tests can only provide
supplementary information’s to confirm COVID-19 infection.

5.3.1. Enzyme-linked immunosorbent assay (ELISA)

Various recombinant antigens such as envelop protein (E), spike protein (S), nucleocapsid protein (N) and membrane protein (M) for COVID-19, are now available on GeneMedi, and they made the antigen detection possible through the different serological assays.

As a result, different ELISA kits based on specific antibodies (i.e. IgG and IgM) can also be used for detecting COVID-19. Zhong et al., (2020) demonstrated that as ELISA is being rationally pronounced throughout its capacity, this technique is considered as one of the efficient tools for detection of the SARS-CoV-2 antigen. The principle of ELISA assay is based on double sandwich of the antigen and antibodies complex. The assay plate is coated with a primary capture antibody that on the addition of a specimen interacts with the antigen, and then a secondary antibody conjugated with biotin acts as a detecting antibody (Zhao et al., 2020), as expressed in Fig. (3).

Fig. 3. Mechanism of ELISA Detection: The capture antibody is fixed on a solid support, the antigen recognizes and then binds with the capture antibody. After that, binding of a secondary antibody along with biotin Ab-Ag-Ab complex is formed. The biotin attached with secondary antibody act as a detector

Liu et al., (2020b) reported that the SARS-CoV-2 ELISA kits are highly based on recombinant nucleocapsid (rN-based) or it may be based on the recombinant spike (rS-based) proteins, which will detect the IgM and IgG antibodies. Liu et al., (2020b) study reported that on detecting the IgM antibodies, the rS-based ELISA kits are considered to be highly sensitive compared to the rN-based ELISA; however, on detecting the IgG antibodies, there is no significant difference observed. In spite of ELISA being a simple, safe and rapid process with maximum specificity, but is effective in late diagnosis only, and no sufficient data is available on the precise serological diagnosis of COVID-19.
5.3.2. Point-of-Care (POC) and other rapid testing’s

A number of POC testing, including lateral flow assays and Loop-mediated isothermal amplification (LAMP) are made available by the different diagnostic test manufacturers, but the WHO recommended its use only in research settings (WHO, 2020a).

However, recent studies of FDA. (2020a); Li et al., (2020) reported that under emergency use authorization (EUA) approval by the FDA, a variety of newly developed combined IgM-IgG antibody-based tests have attained remarkable attention for fast screening of COVID-19 (Fig. 4).

Fig. 4. Demonstration of different testing results through lateral flow assay, including a control line (C), IgM line (M) showing recent infection, and IgG band indicating early exposure to the virus

Another recent breakthrough in the *in vitro* diagnostic (IVD) assays is the development of ID Now™ COVID-19 test by Abbott, (2020a), which targeted the RdRp gene of the SARS-CoV-2, thus provided a leading molecular POC platform in the US. Recently, Abbott, (2020a), (2020b); FDA. (2020b) reported that this POC assay utilizes an isothermal amplification technology for quantitative detection of the viral RNA in a time limit of 5 minutes.

Due to the competition of speedy testing discovery by different manufacturers, Colloidal Gold- Immuno-chromatographic Assay (GICA) is another conventional serological assay that developed for rapid diagnosis, and showed maximum sensitivity with 100% specificity for COVID-19. A study conducted by Xiang et al., (2020b) revealed that kits for this GICA assay were commercialized for the 1st time by Zhu Hai Liv Zon Diagnostics Inc. Moreover, Padoan et al., (2020) highlighted that the Chemiluminescence immunoassay (CLIA) is a new alternative IgM and IgG based supplementary serological assay, which uses the antigens from the N and S proteins of the SARS-CoV-2 that are coated on the magnetic bead of the assay.

Further development in the CLIA assay is made by the Diazyme laboratory, USA, which used an automated DZ-Lite 3000 Plus chemi-luminescence analyzer. In addition, they received the FDA emergency approval for the DZ-Lite SARS-CoV-2 CLIA discovery, as recorded by San Diego, (2020).
5.4. CRISPR/Cas detection system

The clustered regularly interspaced short palindromic repeats (CRISPR) is known to be a well-established biotechnological gene-editing technique, recently used in the in vitro detection of nucleotides sequences, hence it may play a dynamic role as an emerging and precise tool for viral diagnosis in molecular biology (Aman et al., 2020). Although the CRISPR/Cas system is a gene-editing tool, it has been reported that apart from the well-known CRISPR/Cas9 system, two other types of CRISPR systems can play significant roles in the detection of SARS-CoV-2 (Guo et al., 2020a). The main components of such CRISPR/Cas diagnostic systems are shown in Fig. (5).

![Fig. 5. CRISPR/Cas diagnostics is based on specific nucleic acid recognition and its cleavage along with non-specified reporter molecule. The gRNA recognizes the target nucleic acid sequence while the activated Cas protein cleaves it. This CRISPR/Cas complex cut the reporter molecules, which in turn emit visual signals acting as a detector for a specified nucleic acid](image)

5.4.1. CRISPR/Cas13a (SHERLOCK)

The combination of the collateral effect of Specific high sensitivity enzymatic reporter unlocking (SHERLOCK) technology with the isothermal amplification led to the discovery of a CRISPR based diagnostic tool, having high sensitivity and single-base specificity, which may provide rapid detection of the nucleic acid (Gootenberg et al., 2017; Kellner et al., 2019). Moreover, Li et al., (2018); Metsky and Freije, (2020) revealed that as SHERLOCK uses RNA based detection system, thus through using the CRISPR-Cas13a based technology, it is now possible to do real-time in vitro detection of the target RNAs. In such case, the phenomenon of signal amplification is based on a non-specific collateral cleavage of adjacent non-targeted reporter RNA (Jia et al., 2020). The mechanism of SHERLOCK CRISPR/Cas model for detection of SARS-CoV-2 as demonstrated in Fig. (6), is based on the use of two particular gRNAs targeting S gene and Orf1ab. In the proposed design, isothermal amplification of the extracted RNA takes place using recombinase polymerase amplification (RPA) kit, which requires 25 minutes of incubation. The pre-amplified RNA sequence is then determined through 30 minutes of incubation with Cas protein. After a final incubation of two minutes, the result can be detected visually using a commercially available dipstick (Guo et al., 2020b).
5.4.2. CRISPR/Cas12a based detector

Recent studies of Broughton et al., (2020); Lucia et al., (2020) proposed another CRISPR/Cas12a based detector, which can be used as a virtuous alternative to the RT-PCR assay for the rapid diagnosis of SARS-CoV-2. The whole mechanism of CRISPR/Cas12a detection is presented in Fig. (7). Guo et al., (2020b) proposed a design for this CRISPR CAS detection through using a combination of CRISPR and nucleic acid amplification methods along with other detection protocols, with reduced cross-reactivity. Since the CRISPR/Cas strategy is less resource-intensive and will take comparatively less time than RT-PCR, therefore it would be highly sensitive and could be more specific for detection of SARS-COV-2, according to LeMieux, (2020); Morales-Narváez and Dincer, (2020). However, the major possible downside of CRISPR/Cas technology will be its off-target effects. Moreover, it is expected that CRISPR/Cas will need a high load for viral detection; hence it may produce slightly more false-negative results than RT-PCR (Jia et al., 2020).

Various other in vitro diagnostic (IVD) companies have developed a large number of rapid testing systems. Being highly sensitive and specific, there is no need for advanced-level equipment for these tests and they can be operated on a large scale. However, related to its accuracy and evaluation, there is very little peer-reviewed data available for such rapid testing assays. As a result, many methods are not recommended by WHO as reliable tools for COVID-19 detection (Mallapaty, 2020; WHO, 2020a, b).
Fig. 7. Schematic flow of CRISPR/Cas12a detection system for SARS-CoV-2: RNA extraction from the patient's sample takes place, after which reverse transcription isothermal amplification of the extracted RNA occurs. The gRNA recognizes the single strand targeted complementary DNA sequence specifically and is degraded by Cas12a, whereas the single-strand DNA probe is cleaved nonspecifically by the Cas12a nucleases. This Cas12a mediated collateral cleavage of the ssDNA probe can be visualized through a color change thus acting as a detector.

**Conclusion**

The present study reviewed the various possible diagnostic tools and compared the significances and limitations of each assay used for COVID-19 detection. Although the most precise and highly specific method for detection of COVID-19 is RT-PCR; however, the current situations also demand new and rapid diagnostic approaches. Therefore, by reviewing the different available detection technologies, and the recent application of CRISPR/Cas based detection in in vitro diagnostics, we concluded that this quick and accurate diagnostic strategy might play a complementary role in the detection of COVID-19. However, the evaluation of each diagnostic technique and approval from the FDA are necessary before practical application.

**Conflict of interest**

The authors declare that there is no conflict of interest.

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**Ethical Approval**

Non-applicable.

**6. References**

Abbott, (2020a). Abbott Launches Molecular Point-of-Care Test to Detect Novel Coronavirus in as Little as Five Minutes. [https://abbott.mediaroom.com/2020-03-27-Abbott-Launches-Molecular-Point-of-Care-](https://abbott.mediaroom.com/2020-03-27-Abbott-Launches-Molecular-Point-of-Care-).
Test-to-Detect-Novel-Coronavirus-in-as-Little-as-Five-Minutes

Abbott, (2020b). ID NOW™ is a leading molecular point-of-care platform in the United States, trusted by hospitals, physician offices, and urgent care clinics nationwide. https://www.alere.com/en/home/product-details/id-now-covid-19.html

Aman, R.; Mahas, A. and Mahfouz, M. (2020). Nucleic Acid Detection Using CRISPR/Cas Biosensing Technologies. ACS Synthetic Biology. https://doi.org/10.1021/acssynbio.9b00507

Andersen, K.G.; Rambaut, A.; Lipkin, W.I.; Holmes, E.C. and Garry, R.F. (2020). The proximal origin of SARS-CoV-2. Nature Medicine. pp. 2-4. https://doi.org/10.1038/s41591-020-0820-9

Bai, Y.; Yao, L.; Wei, T.; Tian, F.; Jin, D.Y.; Chen, L. and Wang, M. (2020). Presumed Asymptomatic Carrier Transmission of COVID-19. JAMA. 323(14): 1406-1407. https://doi.org/10.1001/jama.2020.2565

Beltrán-Pavez, C.; Márquez, C.L.; Muñoz, G.; Valiente-Echeverría, F.; Gaggero, A.; Soto-Rífo, R. and Barriga, G.P. (2020). SARS-CoV-2 detection from nasopharyngeal swab samples without RNA extraction. bioRxiv. https://doi.org/10.1101/2020.03.28.013508

Broughton, J.P.; Deng, X.; Yu, G.; Fasching, C.L.; Servellita, V.; Singh, J.; Miao, X. et al. (2020). CRISPR – Cas12-based detection of SARS-CoV-2. National Biotechnology. 38(7): 870-874. https://doi.org/10.1038/s41587-020-0513-4

Caly, L.; Druce, J.D.; Catton, M.G.; Jans, D.A. and Wagstaff, K.M. (2020). The FDA-approved Drug Ivermectin inhibits the replication of SARS-CoV-2 in vitro. Antiviral Research. 104787. https://doi.org/10.1016/j.antiviral.2020.104787

Caruso, D.; Zerunian, M.; Polici, M.; Pucciarelli, F.; Polidori, T.; Rucci, C. et al. (2020). Chest CT Features of COVID-19 in Rome, Italy. Radiology. 201237. https://doi.org/10.1148/radiol.2020201237

CDC. (2020a). CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. https://www.fda.gov/media/134922/download

CDC. (2020b). Research Use Only 2019-Novel Coronavirus (2019-nCoV) Real-time RT-PCR Primers and Probes. https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html

Chan, J.F.W.; Yip, C.C.Y.; To, K.K.W.; Tang, T.H.C.; Wong, S.C.Y.; Leung, K.H. et al. (2020a). Improved molecular diagnosis of COVID-19 by the novel, highly sensitive and specific COVID-19-RdRp/Hel real-time reverse transcription-polymerase chain reaction assay validated in vitro and with clinical specimens. Journal of Clinical Microbiology. 00310-20. https://doi.org/10.1128/JCM.00310-20

Chan, J.F.W.; Yuan, S.; Kok, K.H.; To, K.K.W.; Chu, H.; Yang, J. et al. (2020b). A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. The Lancet. 395(10223): 514-523. https://doi.org/10.1016/S0140-6736(20)30154-9

Chen, J.; Wu, L.; Zhang, J.; Zhang, L.; Gong, D.; Zhao, Y. et al. (2020a). Deep learning-based model for detecting 2019 novel coronavirus pneumonia on high-resolution computed tomography: a prospective study. medRxiv. 02.25.20021568. https://doi.org/10.1101/2020.02.25.20021568

Chen, N.; Zhou, M.; Dong, X.; Qu, J.; Gong, F.; Han, Y. et al. (2020b). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. The Lancet. 395(10223): 507-513. https://doi.org/10.1016/S0140-6736(20)30211-7

Colson, P.; Rolain, J.M.; Lagier, J.C.; Brouqui, P. and Raoult, D. (2020). Chloroquine and hydroxychloroquine as available weapons to fight COVID-19. International Journal of Antimicrobial Agents. 105932. https://doi.org/10.1016/j.ijantimicag.2020.105932

Ullah et al., 2020

Novel Research in Microbiology Journal, 2020
Corman, V.M.; Landt, O.; Kaiser, M.; Molenkamp, R.; Meijer, A.; Chu, D.K.W. et al. (2020). Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance. 25(3): 1-8. https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045

Cucinotta, D. and Vanelli, M. (2020). WHO Declares COVID-19 a Pandemic. 91(6): 157-160. https://doi.org/10.23750/abm.v91i1.9397

Cui, J.; Li, F. and Shi, Z.L. (2019). Origin and evolution of pathogenic coronaviruses. Nature Reviews Microbiology. 17(3): 181-192. https://doi.org/10.1038/s41579-018-0118-9

East, M.; Committee, I.; Who, E. and Surveillance, G. (2020). Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases.

Fan, L.; Li, D.; Xue, H.; Zhang, L.; Liu, Z.; Zhang, B. et al. (2020). Progress and prospect on imaging diagnosis of COVID-19. Chinese Journal of Academic Radiology. 3(1): 4-13. https://doi.org/10.1007/s42058-020-00031-5

Fang, Y.; Zhang, H.; Xie, J.; Lin, M.; Ying, L.; Pang, P. and Ji, W. (2020). Sensitivity of Chest CT for COVID-19: Comparison to RT-PCR. Radiology. 200432. https://doi.org/10.1148/radiol.2020200432

FDA. (2020a). Coronavirus Disease 2019 (COVID-19) EUA Information. https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization

FDA. (2020b). ID NOW COVID-19 - Letter of Authorization. https://www.fda.gov/media/136522/download

Feigelman, R.; Burdon, G.; Liu, J.; Oliva, A. and Borcherding, A. (2020). High sensitivity detection of coronavirus SARS-CoV-2 using multiplex PCR and a multiplex-PCR-based metagenomic method.

Guo, A.K. and Rana, D.R. (2020). Charting the challenges behind the testing of COVID-19 in developing countries: Nepal as a case study. Biosafety and Health. https://doi.org/https://doi.org/10.1016/j.bsheal.2020.05.002

Gorbalenya, A.E.; Baker, S.C.; Baric, R.S.; de Groot, R.J.; Drosten, C.; Gulyaeva, A.A. et al. (2020). The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nature Microbiology. https://doi.org/10.1038/s41564-020-0695-z

Guo, L.; Ren, L.; Yang, S.; Xiao, M.; Chang, D.; Yang, F.; Dela Cruz, C.S. et al. (2020a). Profiling Early Humoral Response to Diagnose Novel Coronavirus Disease (COVID-19). Clinical Infectious Diseases. https://doi.org/10.1093/cid/ciaa310

Guo, L.; Sun, X.; Wang, X.; Liang, C.; Jiang, H.; Gao, Q.; Dai, M.; Qu, B.; Fang, S. et al. (2020b). SARS-CoV-2 detection with CRISPR diagnostics. BioRxiv. 2020.04.10.023358. https://doi.org/10.1101/2020.04.10.023358

Hani, C.; Trieu, N.H.; Saab, I.; Dangeard, S.; Bennani, S.; Chassagnon, G. and Revel, M.P. (2020). COVID-19 pneumonia: a review of typical CT findings and differential diagnosis. Diagnostic and Interventional Imaging. https://doi.org/10.1016/j.dii.2020.03.014

Haveri, A.; Smura, T.; Kuivanen, S.; Österlund, P.; Hepojoji, J.; Ikonen, N. et al. (2020). Serological and molecular findings during SARS-CoV-2 infection: the first case study in Finland, January to February 2020. Euro Surveillance : Bulletin European Sur Les Maladies Transmissibles = European Communicable Disease Bulletin. 25(11): 2000266. https://doi.org/10.2807/1560-7917.ES.2020.25.11.2000266

Holshue, M.L.; DeBolt, C.; Lindquist, S.; Lofy, K.H.; Wiesman, J.; Bruce, H. et al. (2020). First Case of 2019 Novel Coronavirus in the United States.
Ullah et al., 2020

New England Journal of Medicine. 382(10): 929-936. https://doi.org/10.1056/NEJMoa2001191

Hu, T.Y.; Frieman, M. and Wolfram, J. (2020a). Insights from nanomedicine into chloroquine efficacy against COVID-19. Nature Nanotechnology. 19-21. https://doi.org/10.1038/s41565-020-0674-9

Hu, Z.; Song, C.; Xu, C.; Jin, G.; Chen, Y.; Xu, X. et al. (2020b). Clinical characteristics of 24 asymptomatic infections with COVID-19 screened among close contacts in Nanjing, China. Science China Life Sciences. https://doi.org/10.1007/s11427-020-1661-4

Huang, C.; Wang, Y.; Li, X.; Ren, L.; Zhao, J.; Hu, Y. et al. (2020a). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The Lancet. 395(10223): 497-506. https://doi.org/10.1016/S0140-6736(20)30183-5

Huang, P.; Liu, T.; Huang, L.; Liu, H.; Lei, M.; Xu, W. et al. (2020b). Use of Chest CT in Combination with Negative RT-PCR Assay for the 2019 Novel Coronavirus but High Clinical Suspicions. Radiology. 295(1): 22-23. https://doi.org/10.1148/radiol.2020200330

Jia, F.; Li, X.; Zhang, C. and Tang, X. (2020). The expanded development and application of CRISPR system for sensitive nucleotide detection. Protein and Cell. 1-6. https://doi.org/10.1007/s13238-020-00708-8

Juurlink, D.N. (2020). Safety considerations with chloroquine, hydroxychloroquine and azithromycin in the management of SARS-CoV-2 infection. Canadian Medical Association Journal. pp. 1-4. https://doi.org/10.1503/cmaj.200528

Kellner, M.J.; Koob, J.G.; Gootenberg, J.S.; Abudayyeh, O.O. and Zhang, F. (2019). SHERLOCK: nucleic acid detection with CRISPR nucleases. Nature Protocols. 14(10): 2986-3012. https://doi.org/10.1038/s41596-019-0210-2

Khan, S.; Siddique, R.; Shereen, M.A.; Ali, A.; Liu, J.; Bai, Q. et al. (2020). The emergence of a novel coronavirus (SARS-CoV-2), their biology and therapeutic options. Journal of Clinical Microbiology. pp. 1-22. https://doi.org/10.1128/jcm.00187-20

Gootenberg, G.S.; Mazutis, L.; Weitz, D.A.; Kirschner, M.W.; Klein, A.M., Mazutis, L. et al. (2017). Nucleic acid detection with CRISPR-Cas13a/C2c2. CRISPR TECHNOLOGY. https://doi.org/10.1126/science.aam9321

LeMieux, J. (2020). COVID-19 Drives CRISPR Diagnostics: CRISPR’s role in DNA detection, not editing, may fill the gap in COVID-19 testing. Genetic Engineering and Biotechnology News. 40(5): 21-22.

Li, Z.; Yi, Y.; Luo, X.; Xiong, N.; Liu, Y.; Li, S. et al. (2020). Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. Journal of Medical Virology. https://doi.org/10.1002/jmv.25727

Li, S.Y.; Cheng, Q.X.; Li, X.Y.; Zhang, Z.L.; Gao, S.; Cao, R.B. et al. (2018). CRISPR-Cas12a-assisted nucleic acid detection. In Cell Discovery. 4(1): 18-21. https://doi.org/10.1038/s41421-018-0028-z

Lin, Q.; Zhao, S.; Gao, D.; Lou, Y.; Yang, S.; Musa, S.S. et al. (2020). A conceptual model for the coronary disease 2019 (COVID-19) outbreak in Wuhan, China with individual reaction and governmental action. International Journal of Infectious Diseases. 93: 211-216. https://doi.org/10.1016/j.ijid.2020.02.058

Liu, W.; Liu, L.; Kou, G.; Zheng, Y.; Ding, Y.; Ni, W. et al. (2020a). Evaluation of Nucleocapsid and Spike Protein-based ELISAs for detecting antibodies against SARS-CoV-2. Journal of Clinical Microbiology. https://doi.org/10.1128/jcm.00461-20

Liu, Y.; Gayle, A.A.; Wilder-Smith, A. and Rocklöv, J. (2020b). The reproductive number of COVID-19 is higher compared to SARS coronavirus. Journal of Travel Medicine. 27(2): 1-4. https://doi.org/10.1093/ijtm/taaa021
Long, C.; Xu, H.; Shen, Q.; Zhang, X.; Fan, B.; Wang, C. et al. (2020). Diagnosis of the Coronavirus disease (COVID-19): rRT-PCR or CT? European Journal of Radiology. 126: 108961. https://doi.org/https://doi.org/10.1016/j.ejrad.2020.108961

Lu, R.; Zhao, X.; Li, J.; Niu, P.; Yang, B.; Wu, H. et al. (2020). Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. The Lancet. 395(10224): 565-574. https://doi.org/10.1016/S0140-6736(20)30251-8

Lucia, C.; Federico, P.B. and Alejandra, G.C. (2020). An ultrasensitive, rapid, and portable coronavirus SARS-CoV-2 sequence detection method based on CRISPR-Cas12. BioRxiv. 2020.02.29.971127. https://doi.org/10.1101/2020.02.29.971127

Mallapaty, S. (2020). Will antibody tests for the coronavirus really change everything? https://doi.org/10.1038/d41586-020-01115-z

Metsky, H.C. and Freije, C.A. (2020). CRISPR-based surveillance for COVID-19 using genomically-comprehensive machine learning design. 1-11.

Morales-Narváez, E. and Dincer, C. (2020). The impact of biosensing in a pandemic outbreak: COVID-19. Biosensors and Bioelectronics. 163: 112274. https://doi.org/10.1016/j.bios.2020.112274

Mungmunpuntipantip, R. and Wiwanitkit, V. (2020). Uncertainty in using chest computed tomography in early coronavirus disease (COVID-19). Canadian Journal of Anesthesia. https://doi.org/10.1007/s12630-020-01639-y

NCBI. (2020). SARS-CoV-2 (Severe acute respiratory syndrome coronavirus). https://www.ncbi.nlm.nih.gov/genbank/sars-cov-2-seqs/

Owens, B. (2020). Excitement around hydroxychloroquine for treating COVID-19 causes challenges for rheumatology. The Lancet Rheumatology. 9913(20): 30089. https://doi.org/10.1016/S2665-9913(20)30089-8

Padoan, A.; Cosma, C.; Sciakovelli, L.; Faggian, D. and Plebani, M. (2020). Analytical performances of a chemiluminescence immunoassay for SARS-CoV-2 IgM/IgG and antibody kinetics. Clinical Chemistry and Laboratory Medicine (CCLM). 20200443. https://doi.org/https://doi.org/10.1515/cclm-2020-0443

Pal, M.; Berhanu, G.; Desalegn, C. and Kandi, V. (2020). Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2): An Update. Cureus. 2(3). https://doi.org/10.7759/cureus.7423

San Diego, C. (2020). Diazyme Laboratories, Inc. Announces Availability of COVID-19 Antibody Tests. http://www.diazyme.com/diazyme-laboratories-announces-availability-of-covid-19-antibody-tests

Shereen, M.A.; Khan, S.; Kazmi, A.; Bashir, N. and Siddique, R. (2020). COVID-19 infection: Origin, transmission, and characteristics of human coronaviruses. Journal of Advanced Research. 24: 91-98. https://doi.org/10.1016/j.jare.2020.03.005

Singhal, T. (2020). A Review of Coronavirus Disease-2019 (COVID-19). Indian Journal of Pediatrics. 87(4): 281-286. https://doi.org/10.1007/s12098-020-03263-6

Sun, C.; Chen, L.; Yang, J.; Luo, C.; Zhang, Y.; Li, J. et al. (2020). SARS-CoV-2 and SARS-CoV Spike-RBD Structure and Receptor Binding Comparison and Potential Implications on Neutralizing Antibody and Vaccine Development. BioRxiv. 2020.02.16.951723. https://doi.org/10.1101/2020.02.16.951723

Udugama, B.; Kadhiresan, P.; Kozlowski, H.N.; Malekjahani, A.; Osborne, M.; Li, V.Y.C. et al. (2020). Diagnosing COVID-19: The Disease and Tools for Detection. ACS Nano. https://doi.org/10.1021/acsnano.0c02624

Wang, C.; Pan, R.; Wan, X.; Tan, Y.; Xu, L.; Ho, C.S. and Ho, R.C. (2020a). Immediate psychological responses and associated factors during the initial
stage of the 2019 coronavirus disease (COVID-19) epidemic among the general population in China. International Journal of Environmental Research and Public Health. 17(5). https://doi.org/10.3390/ijerph17051729

Wang, L.; Wang, Y.; Ye, D. and Liu, Q. (2020b). A review of the 2019 Novel Coronavirus (COVID-19) based on current evidence. International Journal of Antimicrobial Agents. 105948. https://doi.org/https://doi.org/10.1016/j.ijantimicag.2020.105948

WHO. (2020c). Advice on the use of point-of-care immunodiagnostics tests for COVID-19. https://www.who.int/news-room/commentaries/detail/advice-on-the-use-of-point-of-care-immunodiagnostic-tests-for-covid-19

WHO. (2020). WHO Director-General’s opening remarks at the media briefing on COVID-19. https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19

Wu, Y.C.; Chen, C.S. and Chan, Y.J. (2020). The outbreak of COVID-19: An overview. Journal of the Chinese Medical Association. 83(3). https://journals.lww.com/jcma/Fulltext/2020/03000/The_outbreak_of_COVID_19_An_overview.3.aspx

Xiang, J.; Yan, M.; Li, H.; Liu, T.; Lin, C.; Huang, S. and Shen, C. (2020b). Evaluation of Enzyme-Linked Immunoassay and Colloidal Gold-Immunochromatographic Assay Kit for Detection of Novel Coronavirus (SARS-CoV-2) Causing an Outbreak of Pneumonia (COVID-19). MedRxiv, 2020.02.27.20028787. https://doi.org/10.1101/2020.02.27.20028787

Zhang, F.Y.; Qiao, Y. and Zhang, H. (2020). CT imaging of the COVID-19. Journal of the Formosan Medical Association. https://doi.org/https://doi.org/10.1016/j.jfma.2020.04.006

Zhang, Y.Z. and Holmes, E.C. (2020). A Genomic Perspective on the Origin and Emergence of SARS-CoV-2. Cell. https://doi.org/https://doi.org/10.1016/j.cell.2020.03.035

Zhao, J.; Yuan, Q.; Wang, H.; Liu, W.; Liao, X.; Su, Y. et al. (2020). Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Clinical Infectious Diseases. https://doi.org/10.1093/cid/ciaa344
Zhong, L.; Chuan, J.; Gong, B.; Shuai, P.; Zhou, Y.; Zhang, Y. et al. (2020). Detection of serum IgM and IgG for COVID-19 diagnosis. Science China Life Sciences. https://doi.org/10.1007/S11427-020-1688-9