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COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE

DEPARTMENT OF BIOTECHNOLOGY

Review Research Article on:

APPLICATION OF BIOTECHNOLOGY ON DIAGNOSTIC STRATAGES AND VACCINE APPROACH FOR COVID-19

A Review Research paper submitted to Department of Biotechnology for the partial fulfillment of the requirements for BSC, Degree in biotechnology.

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SENIOR PROJECT SUBMISSION FORM

This is to certify that senior project entitled as'' **Application of Biotechnology on Diagnostic Stratages and Vaccine Approach for Covid-19'',** submitted in partial fulfillments for the Bachelor of sciences Degree in Biotechnology by below listed students, complies with the regulations of the university and meets the accepted standard with respect to originality and quality.

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ACEs	Angiotensin-converting enzyme 2
COVID-19	Coronavirus disease 2019
CoVs	Corona virus
CSG	Corona virus study group
CT	Computed tomography
FDA	Food and Drug Administration
gRNAs	guide RNAs
LAMP	Loop-mediated isothermal amplification
MERs	Middle East respiratory syndrome corona virus
NS	Nasopharyngeal swab
RT-PCR	Real time polymerase chain reaction
SARS-CoV-1	Severe acute respiratory syndrome coronavirus 1
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
WHO	World health organization

ABSTRACT

Covid-19 is the viral disease caused by a new coronavirus which was first reported on December 31, 2019 in the Wuhan province of china. It belongs to a large family of respiratory viruses called Coronaviridae and affect both human and animal. It is known to cause severe acute respiratory syndrome (SARS-CoV-2) and Middle East respiratory syndrome (MERS-CoV) and now globally famous novel coronavirus sever acute respiratory syndrome (SARS-CoV-2). The SARS-CoV-2 genome is positive single-stranded RNA (+ssRNA) and have length of 30kbp. There are four genera of coronavirus (alpha, beta, gamma and delta). Among these the novel coronavirus severs acute respiratory syndrome (SARS-CoV-2) belongs to betacoronavirus. In this review, we have focused on the current standard diagnostic approach such as RT-PCR, Immunological technique, CRISPR/Cas, LAMP technique and CT scan. we are also focused on the application of biotechnology for rapid and cost effective diagnostic tools and possible vaccine approach such as protein subunit, inactivated vaccine, vector vaccine and anti- viral drug (Novavax, AstraZeneca, Pfizer, Moderna and Sinopharm), which are currently in use for covid-19 to reduce the spread of the disease. Still the above diagnostic technique has some limitations regarding a lack of strength and lengthy procedures difficult in controlling this outbreak of covid-19. Due to this early detection and isolation of suspected patients can play an essential role in controlling this outbreak.

***Key words: Covid-19, Diagnostic tools, Vaccines**,*

1. INTRODUCTION

Humankind has observed various pandemics throughout the history where some of them were more disastrous than the others to the humans. We are observing a very tough time once again fighting an imperceptible enemy the novel COVID-19 coronavirus**.** COVID-19 initially observed in the Wuhan province of China, in December 2019, now vastly spreading around the world. It is a clustered of pneumonia cases, caused by a newly identified β-coronavirus (We *et al*., 2020). WHO officially named the disease as coronavirus disease 2019 (COVID-19) and Coronavirus Study Group (CSG) of the International Committee proposed to name the new coronavirus as SARS-CoV-2, both issued on 11 February 2020 (WHO, 2020). According to Cleri *et al.*, (2010), twenty-six different species has known and are been divided into four genera (alpha, beta, gamma and delta) which are characterized by different antigenic cross-reactivity and genetic make-up of the known coronavirus species. Only six has been known to cause disease in humans: HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory virus coronavirus (MERS-CoV) (Arabi *et al.,* 2018, Skariyachan *et al.,* 2019). The novel emergent virus named SARS-CoV-2 exhibits a rapid human-to-human spreading via the respiratory tract, and is responsible of a syndrome known as Coronavirus disease of 2019 (COVID19) (Mahase 2020). According to information displayed on the World Health Organization (WHO) COVID-19 Dashboard, COVID-19 has become a pandemic outbreak with more than 212,357,464 peoples as of August 24, 2021, are infected and around 4.5 million peoples were lost their life worldwide by this virus. In Africa as of August 24, 2021, 7.2 million cases were confirmed and 190, 496 were dead. Here in Ethiopia, 297,918 peoples are infected and, 4,580 Ethiopians lost their life by the virus (WHO., 2021).

Different scholars were being reported, SARS-CoV-2 is the beta-coronavirus that originated from bats. These newly emerging virus diseases have become a major public health threat around the world in recent years. During the last two decades, outbreaks of several viral diseases have reported including the severe acute respiratory syndrome coronavirus (SARS-CoV) (*Xu et al*., 2020). In 2002, the Middle East respiratory syndrome coronavirus (MERS-CoV) (zumla *et al*., 2015) in 2012.

The genome of SARS-CoV-2 is positive single-stranded RNA (+ssRNA) and has length of about 30 k nucleotides. It has now been shown that the virus causing COVID-19 is a SARS-like coronavirus that had previously been reported in bats in China. Almost 85% homology of this virus is also similar to the SARS-CoV (petrosillo *et al*., 2020). Four main structural proteins have been encoded in SARS-CoV-2 viral genome, namely, spike (S) surface glycoprotein, membrane (M) protein, envelope (E) protein, and nucleocapsid (N) protein. The replication of COVID-19 as any other virus requires a host cell and normally includes attachment, penetration, uncoating, replication, assembly, and release steps (Goldman-Israelow, 2020). SARS CoV-2 propagates through RNA replication using RNA dependent RNA polymerases enzyme. This virus can mutate slowly, posing a challenge for its treatment and control. The symptoms of COVID -19 may arise within 2 to 14 days after the infection. Besides, in some cases, the diseases prevail after 27 days. However, Chinese researcher s mentioned 5.2 days as an average incubation period (Li *et al.,* 2020). The duration of the survival of death is 6 to 41 days after infection of the coronavirus. It depends on the age, health and clinical conditions of the patients (Wan *et al.,* 2020).

Several current reports were proposed the use of natural substances as therapeutic antiviral against COVID-19 that have demonstrated efficacy against other coronaviruses related with SARS-CoV-2. Another strategy for the systematic fight against the novel COVID-19 pandemic resides in the prevention of infection through immunization of uninfected individuals with effective vaccine to induce protective anti-SARS-CoV-2 immune responses and long-lasting memory. In this sense, Nanotechnology and new materials are other open fronts in the prevention, detection and vaccine approach against COVID-19 disease. According to WHO report, the pandemic is still increasing in different part of the world countries, particularly the pandemic is highly accelerating in developing countries. Currently there are different application of biotechnology for effective diagnostics strategies and vaccine approach for covid-19. Therefore, it should have been known around the world to control this rapid spread of the pandemic. In this review, we have focused on the application of biotechnology for rapid and cost effective diagnostic tools and possible vaccine approach, which are currently in use for covid-19 to reduce the spread of the disease.

2. ORIGINS OF CORONAVIRUS DISEASE-19 (COVID-19)

The current pandemic, COVID-19, have believed to have emerged from an animal host. The COVID-19 is caused by the SARS- CoV-2 virus, whose genome shares 96% similarity with betacoronavirus isolated from a bat in 2013 (Wong *et al.*, 2020). The sequence of the receptor-binding motif (RBM) of SARS-CoV-2, which is critical for host infection, also shares a high-sequence similarity with the betacoronavirus isolated from a Malayan pangolin (Wong *et al*., 2020). In fact, the percentage of bases identical to the SARS-CoV-2 RBM sequence is higher for pangolin-CoV (98.7%) than for bat (77.6%). Wong *et al,* (2020) observed that some of the amino acids shared uniquely between SARS-CoV-2 and pangolin-CoV occur at the key sites engaged in host binding. Therefore, it has been speculated that SARS-CoV-2 originated in bats and went through multiple recombination events as it migrated through other mammals. In addition to Bat, the novel coronavirus originated from the human seafood market at Wuhan, China where snakes, raccoon dogs, palm civets, and other animals sold (Shereen *et al*., 2020.

3. STRUCTURE AND REPLICATIONS CYCLES OF CORONA VIRUS

Replication is the process of duplicating or producing an exact copy of a polynucleotide strand such as DNA and RNA. The replication of COVID-19 as any other virus requires a host cell and normally includes attachment, penetration, uncoating, replication, assembly, and release steps (Goldman-Israelow, 2020). Spike glycoprotein on the surface of the COVID-19 binds to angiotensin-converting enzyme 2 (ACE2) receptor protein located on the host cell plasma membrane and facilitates the host cell invasion. Serine protease produced by a host cell facilitates the process of this invasion. After receptor-mediated endocytosis of the virus into the host cells, it releases viral genome (single-stranded positive RNA), and using host ribosome translates into viral polyproteins. Viral proteinases cleave viral polyproteins into effector proteins. RNA-dependent RNA polymerase in turn synthesizes a full-length negative-strand RNA template, which is used to make more viral genomic, RNA Viral genome, then, is synthesized by genomic replication. There are four essential structural viral proteins namely;

spike (S) surface glycoprotein, membrane (M) protein, envelope (E) protein and nucleocapsid (N) protein are produced by transcription then translation. As indicated on fig.1 N protein binds genomic RNA while, S M, and E proteins are integrated into the membrane of the endoplasmic reticulum (ER), forming endoplasmic Reticulum-Golgi intermediate compartment (ERGIC).

Assembled nucleocapsid with helical twisted RNA was been encapsulated into the ER lumen, viral progeny is transported by the ERGIC toward the plasma membrane of the host cell, and finally, daughter virus is released by exocytosis. Fig1: shows the replication cycle of coronavirus pandemic.

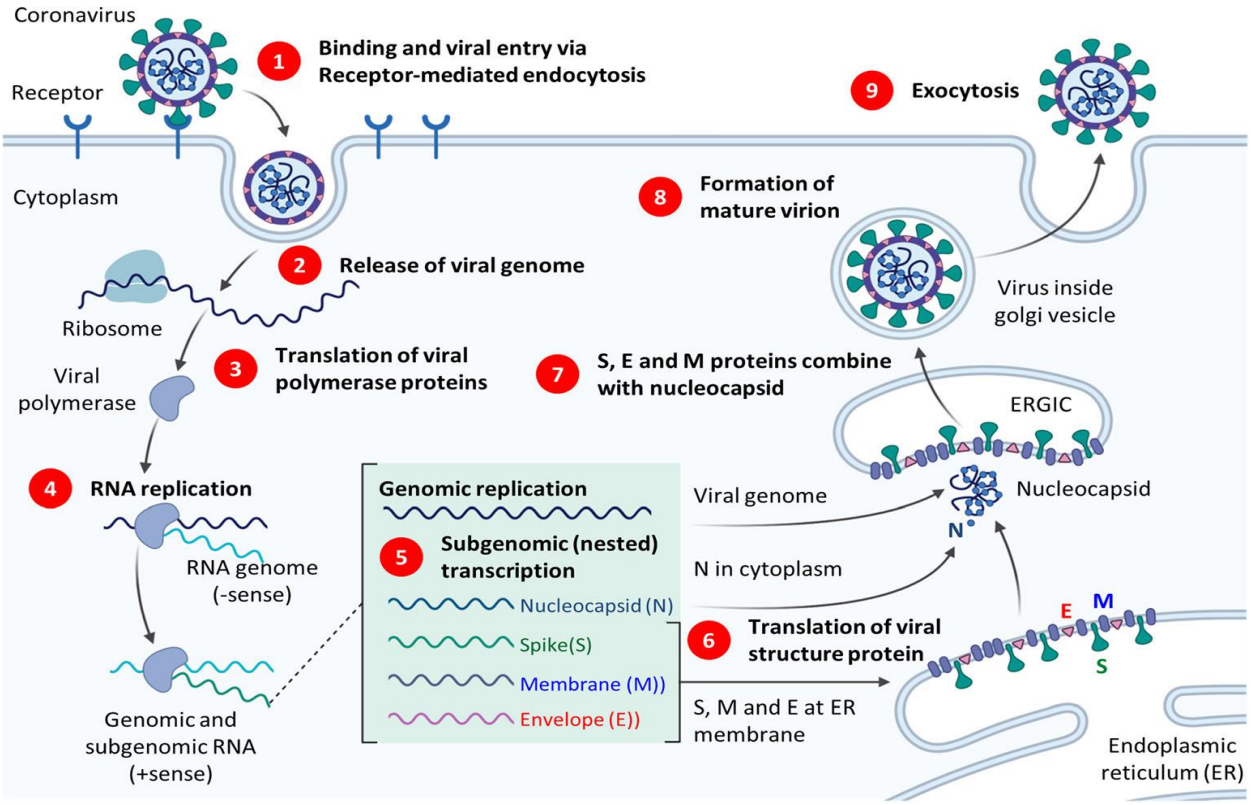

Figure 1 Figure 3: Summary of the various approaches towards diagnosis of SARS CoV-2 infection

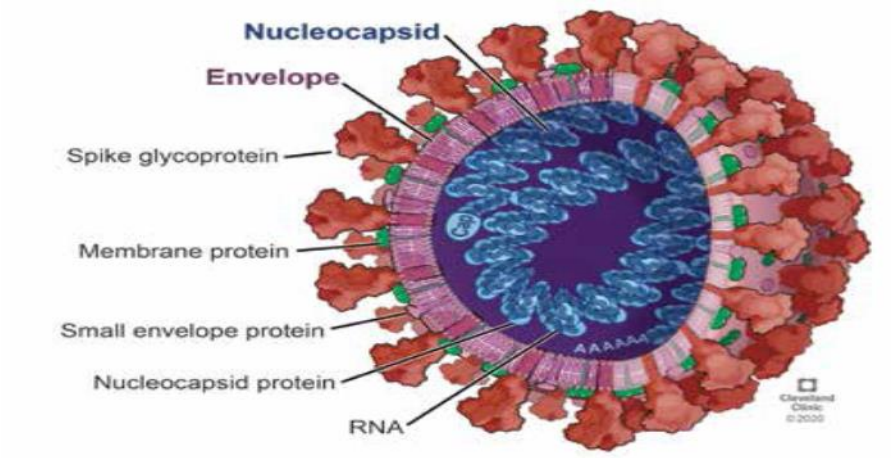

Figure 2: Structure of corona viruses (Fehr and Perlman,2020)

4. THE EPIDEMIOLOGICAL CHARACTERISTICS OF SARS-COV-2 AND OTHER CORONAVIRUSES

Coronaviruses contain a positive-sense single-stranded RNA (+ssRNA) enclosed in a capsid with spikes, which resemble solar corona. Relative to other positive RNA viruses, coronaviruses have a large genome and possess sophisticated machinery to overtake host cells. They are known to cross species barriers, infect humans, and hijack the host cells to replicate further and spread. As of now, there are some effective means of prevention or treatments against corona viruses, which have become a significant source of respiratory disease outbreaks. Four of the six corona viruses that were previously known to infect humans cause common colds upper respiratory and intestinal illnesses. Of these, beta-coronaviruses like SARS-CoV-2 and Middle East respiratory syndrome coronavirus (MERS-CoV) cause severe and often fatal lower respiratory tract infections (de Wilde *et al*., 2017). The new corona virus, which was known as, Delta, or B.1.617.2, was first identified in India in Dec. 2020. Within a matter of months, this particular variant spread to over 98 countries around the world, becoming the dominant variant in more than a dozens of those countries, including India, the U.K., Israel and the United States. Delta is now responsible for more than 83% of COVID-19 cases being reported in the U.S. and, with conditions are ripe for continued evolution and spread of SARS-CoV-2. Data indicate that Delta is 40-60% more transmissible than Alpha and almost twice as transmissible as the original Wuhan strain of SARS-CoV-2. (Campbell et al., 2021, Stirrup *et al.*, 2021)

Table 1: Comparison of epidemiological characteristics between different types of Corona virus

Features	Types of Corona Virus						
	SARS-CoV-2	MERS-CoV	SARS-CoV	HCoV-229E	HCoV-NL63	HKU1	HCoV-OC43
Host of virus	Bats are natural hosts, pangolins are Intermediate hosts, and humans are terminal hosts	Bats are natural hosts, dromedary camels are intermediate hosts, and humans are terminal hosts	Chinese horseshoe bats are natural hosts, masked palm civets are intermediate hosts, and humans are terminal hosts	Bat are natural host, Camelids are Intermediate host and human are terminal host	Bat are natural host, palm civets are Intermediate hosts and human are terminal hosts	Swine are natural host and human are terminal host	Antelops are natural host, dogs are Intermediate hosts and humans are terminal host
Transmission mode	Human-to-human through fomites, physical contact, aerosol droplets, nosocomial transmission,	Human to human contact, through respiratory secretion such as coughing.	Through human to human contact and droplet transmission(aerosol), through fomite transmission	Direct human to human contact	Through droplets transmitted such as cough, breath and sneezes	Human to human contact, through droplets	Human to human contact through droplets

	zoonotic transmission						
Incubation period	6.4days(range: 0-24 days)	2-14 days	2-7 days	2-5 days	7-10 days	6-8 days	2-4 days
Reference	**Cui *et al.*, 2019**	Guen *et al.*, 2003	Chan, Lau and Woo, 2013	Perlman And Netland, 2009	Wege and Termeulen, 1982	Richard *et al.*, 2017	Alekseev *et al* ., 2008

5. APPLICATION OF BIOTECHNOLOGY FOR COVID-19 PANDEMIC

Today, there are several applications of biotechnology for COVID-19 pandemic in different aspects, such as treatment, diagnosis and prevention. Currently, several vaccines have been administered to the world population. The use of different vaccines directed against COVID-19, such as inactivated vaccines, protein subunit, nucleic acid-based vaccines and vector vaccines. The first mass vaccination program was launched in December 2020. In fact, on 31 December the WHO organization approved the use of the Pfizer (Biotech Company) which produce mRNA vaccine for COVID-19 with an efficiency of 95–96%. Later on, another mRNA vaccine, commonly known as Moderna, was approved in January 2020. Finally, the latest to be approved are the vector vaccines known as AstraZeneca and Johnson and Johnson that produce adenovirus vaccine. One of the key questions today is whether vaccines could preserve against infections from new SARS-CoV-2 variants or note (Fontanet *et al*., 2021). Furthermore, AstraZeneca was observed to show 83% efficiency for the UK variant but only 22% for the South African variant. Although vaccines currently in use remain the only effective weapon for the prevention of COVID-19 it appears that they do not allow full coverage for existing variants which is why it is

important to focus with the same attention on new therapeutic approaches for the treatment of COVID-19 (Cascella *et al*., 2021).

Currently, there is various diagnostic techniques for detection of covid-19 such as the reverse transcription polymerase chain reaction (RT-PCR) which is the gold standard test for the diagnosis of COVID-19 (Udugama *et al*., 2020), LAMP is a diagnostic technique that is comparatively less expensive, much sensitive and rapid than RT-PCR and Serological test which involves analysis of blood serum or biological fluids. The other approach of biotechnology on Covid-19 is treatment and vaccination. Different anti-viral and vaccine are available for covid-19 treatment. To manage COVID-19, it should depend upon the timely diagnosis and standard) treatments (Humazah *et al.,* 2020).

6. EMERGING RAPID AND COST EFFECTIVE DIAGNOSTIC TOOLS

COVID-19 has spread massively all over the world since its breakthrough making the field of disease detection and testing to revolutionize (Udugama *et al*., 2020). Enormous biological examination is done attributed to the structure of virus this is help in development of both the vaccine and the diagnostics measures further to constrain the spread of virus. Here in we elaborate specifically on nucleic acid, RT PCR, LAMP-based, CRISPR-BASED, (CT) scan, etc (Leeflang *et al*., 2013). Protein based diagnostic techniques developed over time for detection of COVID-19.

Clinical diagnostic testing plays an essential role in the clinical care of patients with infectious diseases. Early diagnosis and isolation of suspected patients play a vital role in controlling this outbreak (Novel CPERE., 2020). This includes the detection of specific pathogens and monitoring of patient conditions, decisive therapy, measuring prognosis, management and disease surveillance. Various laborator techniques have been used to confirm the presence or absence of the virus as well as determining its severity. The specificity and sensitivity of different diagnostic techniques differ between populations and types of equipment (Leeflang *et al*., 2013). The clinical diagnosis of COVID-19 is focused primarily on epidemiological data, clinical symptoms, and some adjuvant technologies, such as nucleic acid detection and immunological assays. In addition, the isolation of SARS-CoV-2 requires high throughput

equipment (biosafety level-3) to ensure personnel safety (Caruana *et al*., 2020). Currently several diagnostic approaches are used for detection of coronaviruses (SARS-CoV-2).

6.1. REAL TIME- PCR (RT-PCR)

Real time reverse transcription polymerase chain reaction (real time RT-PCR) is a nucleic acid-based technique, as the frontline diagnostic approach to detect SARS-CoV-2 infection in suspected patients. Currently, the reverse transcription-polymerase chain reaction (RT-PCR) is the gold standard test for the diagnosis of COVID-19 (Udugama *et al., 2020*). Mostly nasal swab and ocular secretions samples are being used in the TR-PCR test (Xia *et al*., 2020; Team *et al*., 2020). The aims of real-time RT-PCR are to perform early, rapid and accurate diagnostics and also guide patient care and management as well as epidemiological strategies. The molecular diagnosis nowadays mainly relies on real-time RT-PCR techniques, which are considered reference ones, as they present high sensitivity and specificity and are compatible with automation (Caruana *et al*., 2020). In the first published reports on applying the RT-PCR in the COVID-19 diagnosis, targeting the spike gene region (S) of SARS-COV-2 has shown remarkable specificity and limited sensitivity (Corman *et al*.,2019). One of the earliest workflows set up in response to the outbreak was by Christian Dorsten and colleagues (Corman *et al*., 2020). The team established an accurate, sensitive, and specific RT-PCR protocol against the SARS-CoV-2. Later research, including a comparative study by Nalla *et al*, (2020) have attested to its superior sensitivity (Nalla *et al*., 2020). A notable feature of this pioneering work was that the assays were designed without access to any actual SARS-CoV-2 genomic specimens or patient samples, by relying on genome information sourced from Chinese researchers and synthetic nucleic acid technology. Most diagnostic devices relying on RT-PCR-based amplification suffer from the drawback of requiring either sample lysis or purified nucleic acid samples, a step that involves additional reagents, increased testing time, human errors and costs. Generally, RT-qPCR has a high specificity as a gold standard assay for the final diagnosis of COVID-19. However, its sensitivity could be variable based on viral load, RNA extraction technique, sampling source, and disease stage during the time of sampling. Indeed, the RT-PCR false-positive results are related to the cross-contamination of samples and handling errors (Oliveira *et al*.,2020).

6.2. LAMP-BASED TECHNIQUE

The Loop-mediated isothermal amplification (LAMP) is a new isothermal nucleic acid amplification method with great efficiency. This is used to amplify RNAs and DNAs with high specificity and sensitivity due to its exponential amplification feature and 6 particular target sequences diagnosed by 4 separate primers (Notomi *et al*., 2000). The LAMP assay is rapid and does not need high-priced reagents or equipment. LAMP is a diagnostic technique that is comparatively less expensive, much sensitive and rapid than RT-PCR. This technique involves the selective amplification of target nucleic acids at a constant temperature, usually 60°C. In this technique 4 to 6 specifically designed primers are used to detect distinct nucleic acid sequences, moreover there is no requirement of initial template denaturation and reaction time is minimized up to 30 minutes using strand-displacement polymerases (Kashir and Yaqinuddin, 2020). The Loop-mediated isothermal amplification (LAMP) utilizes the DNA polymerase and 4 to 6 different primers binding to the distinct sequences on the target genome (Bernheim *et al.*, 2029). In the LAMP reactions, the amplified DNA is indicated by turbidity arising from a by Product of the reaction, detectable color generated by a pH-sensitive dye, or fluorescence produced by a fluorescent dye (Udugama *et al*., 2020). The approach occurs at a single temperature, in less than one hour, and with minimal background signals. The LAMP diagnostic testing for COVID-19 is more specific and sensitive compared with the conventional RT-PCR assays and do not dependent on specialized laboratory equipment such as a thermocycler. However, due to the multiplicity of primers used in this method, optimizing the reaction conditions is a major challenge (Shen *et al*., 2020; Yang *et al*., 2020). Lin Yu *et al*. have been used Combines reverse transcription with LAMP diagnostic technique (RT-LAMP) allowing direct detection of SARS-CoV-2 RNA.

This technique was further coupled with a pH indicator, which helped in visual read out of the amplification reaction via color change in the reaction mixture (Yu *et al*., 2020). RT-LAMP based assay that targets spike (S) gene of SARS-CoV-2 developed by Hu *et al* (2020) which showed 88.89% sensitivity and high consistency compared with RT-PCR based diagnostic methods. Time required for this assay was around an hour, considerably lower than RT-PCR (Hu *et al*., 2020). LAMP technique avoids the use of costly reagents and instruments, thus helping in reducing the cost of diagnosis with rapid results (Cascella *et al*., 2020). Various studies have

highlighted the application of LAMP technique in detecting coronavirus infections in patient samples (Poon *et al*., 2004; Pyrc *et al*., 2011).

6.3. COMPUTED TOMOGRAPHY (CT) SCAN

CT Scan is also one of the diagnosis techniques having high sensitivity due to which many researchers recommend its use as one of the necessary auxiliary diagnostic method for COVID-19. Typical features by CT of COVID-19 patient include bilateral multi-lobar ground-glass specificities with differently distributions in posteriors and also in peripheral (Pan et al., 2020), According to a recent report from Wuhan, the CT is significantly more sensitive than PCR for SARS-CoV-2 suspected persons. The results concluded that in patients having negative RT-qPCR reports, more sensitive and accurate conclusion can be achieved using a combination of CT-Scan and other standard techniques like RT-qPCR or other sensitive diagnostic tests.

6.4. IMMUNOLOGICAL DIAGNOSIS

Antigen detection and immunological techniques can be used for a rapid and cost-effective diagnosis while providing an alternative to molecular methods. These immunological methods are simple to operate but have low specificity/sensitivity. In the case of COVID-19, virus morphology can be observed by electron microscopy (Guarner, 2020; Hageman, 2019; Pediatric, 2020). Serological test, which involves analysis of blood serum or biological fluids to identify the presence of certain biomarkers such as antibody has generally used to monitor the progress of the disease (Ahmed *et al*., 2020; Casadevall and Pirofski, 2020). Various serological tests such as enzyme-linked immune sorbent assay (ELISA) have been explored to identify the people who have developed antibodies against SARS-CoV-2 virus infection (Nuccetelli *et al*., 2020; Bryant *et al*., 2020). ELISA immunoassays are developed: detecting of COVID-19 antigens or antibodies against virus antigens. These assays are similar and differ in coating of a micro well plate. When it is coated with antigens, it is used for the detection of antibodies generated against the virus. Vice versa, coating with antivirus antibodies allows for the detection of the viral antigens. Recently, the FDA also authorized for a lateral flow serological immunoassay, namely, qSARS-CoV-2 IgG/IgM rapid test, manufactured by Cellex Inc. (Li, 2020: Guo *et al*., 2020).

6.5. CRISPR-BASED DIAGNOSIS

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) has emerged as a game changer in the molecular biology experiments. Nucleic acid detection with CRISPR-Cas13a/C2c2 is a highly rapid, sensitive and specific molecular detection platform, which may aid in the epidemiology, diagnosis, and control of the disease (Gootenberg *et al*., 2017; Wright *et al*., 2016). The CRISPR/Case system, as a potent technology for genome editing, (Qin *et al*., 2017) has recently been transformed into virus detection (He *et al*., 2020). Elements of this system include Case proteins, guide RNAs (gRNAs), and target RNA/DNA fragments. Both Cas13a and Cas12a have collateral cleavage activity after target binding, where nonspecific single-stranded RNA or DNA labeled with a fluorescent reporter and quencher are digested and released, respectively (Qin *et al*., 2019). It has also reshaped the diagnostics of the current time. CRISPR are the DNA sequences found in bacteria and archaea that have been extensively used in gene editing experiments. They play an important role in antiviral defense as the sequences are derived from bacteriophages which have previously infected bacteria (Barrangou, 2015). Lots of CRISPR based techniques are currently in use or have a potential to be an option of point of care testing in pathogen diagnosis like SARS- CoV-2.

CRISPR has the benefit of being used as a Point of Care diagnosis for pathogenic diseases like COVID-19, owing to its simple use and visual detection by fluorescence or color change (Ding *et al*., 2020). Broughton and group had come up with a CRISPR Cas12 based detection method which they claim to be the most rapid (<40) minute technique among isothermal nucleic acid based POC technique. This CRISPR Cas12-based technique is used to detect SARS-CoV-2 from extracted RNA samples of suspected patients (Chen *et al*., 2018; Chiu, 2018). This technique has been designed to perform simultaneous reverse transcription and isothermal amplification using RT-LAMP to the RNA extracted from nasopharyngeal swabs followed by Cas12-mediated detection of virus. The technique has been named DNA endonuclease targeted CRISPR Trans reporter (DETECTR) assay (Broughton *et al*., 2020).

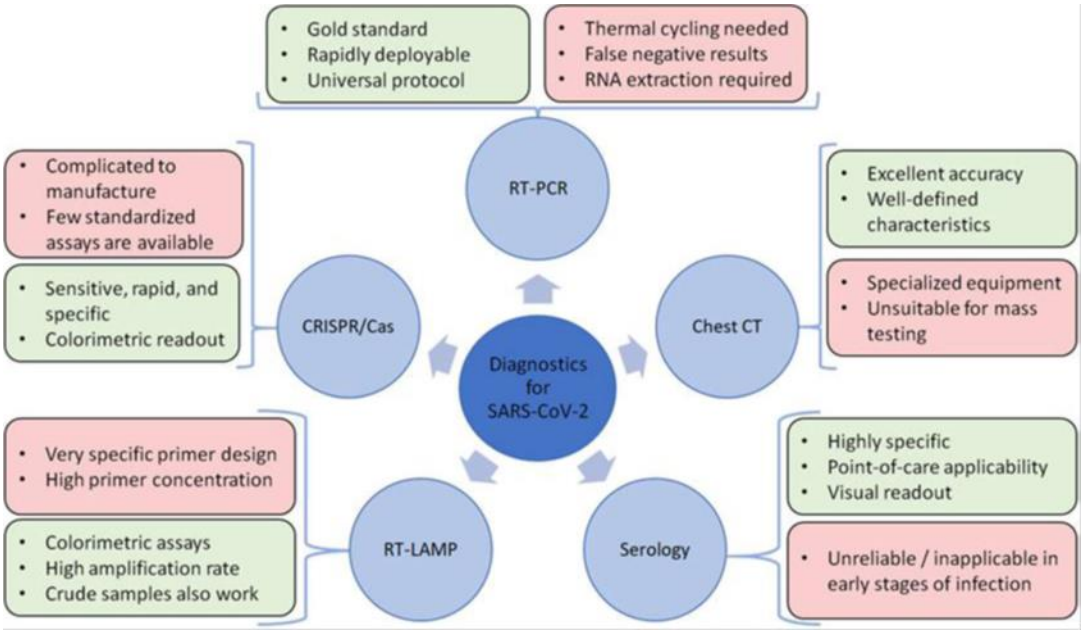

Figure 3: Summary of the various approaches towards diagnosis of SARS CoV-2 infection (M Sreepadmanabh *et al*., 2020)

Table 2 Types of Diagnostic Tools for Coronavirus Disease (COVID-19)

Test types	Mechanism of detection	Testing materials	Positive test indicate	Strong side	Week side	Reference
Real time-PCR (RT-PCR)	Nucleic acid (DNA/RNA)	Nasal swab and ocular secretion	Presence of disease (covid-19)	Fast results, higher sensitivity and need small amounts of DNA	Expensive lab equipment Detection is also complex and time consuming	Xia *et al*., 2020
LAMP-based technique	Use specially designed primer	Samples from nose or throat using swab, mucus produced from hard coughing	Presence of disease (covid-19)	Less expensive, much sensitive and rapid than RT-PCR	Too sensitive, highly prone to false positives due to carry-over or cross-contamination	Shen *et al*., 2020; Yang et *al*., 2020
Computed tomogram ph. (CT) scan	Chest imaging by x-ray	Lung	Presence of disease (covid-19)	More sensitive and accurate than RT-PCR and LAMP methods	Require specialized equipment and not applicable for mass testing	Pan *et al*., 2020
Immunological diagnosis	Antigen-Antibody reaction	Antigen and antibody	Presence of disease (covid-19)	Simple to operate and inexpensive	Have low sensitivity and specificity	Guarner, 2020; Hageman, 2019; Pediatric, 2020
CRISPR-BASED DIAGNOSIS	Collateral cleavage activity of cas endonuclease	Nucleic acid	Presence of disease (covid-19)	a highly rapid, sensitive and specific molecular detection	Complicated to manufacture, few standardized assay are available	Gootenberg *et al*., 2017; Wright *et al*., 2016

7. CURRENT VACCINE DEVELOPMENTS FOR COVID-19

Vaccines are biological preparations that, when administered to an individual stimulate the production of antibodies and provide immunity against one or several specific entities that can cause a disease, such as viruses or bacteria. The vaccines is the most important to the immune system to creating a form of memory allowing an individual to respond faster and with higher extent to a threat when compared with the first time response. Proteins, nucleic acids (DNA and RNA), or even entire organisms can be used for vaccination often in combination with adjuvants that can boost their potency. From an individual point of view, the goal of vaccination is to prevent or modify disease. From a public health perspective, the goal of vaccination is to limit the spread of a pathogen within a population (Graham, 2020). The clinical impact of major scientific advances is also amply evident in the current efforts to rapidly develop an effective vaccine against COVID-19. The advances have enabled shortening of the typical time required for developing an effective vaccine from more than a decade to anticipated less than a couple of years. Currently More than 200 clinical trials are underway to test various novels and repurposed compounds against COVID-19. However, the promise of vaccines is interesting as they have the potential to prevent disease transmission in a larger population. The most promising of these technologies could be broadly classified as protein subunit vaccines, anti-viral drug, inactivated vaccines, viral vector vaccines, and DNA vaccines (Kalita *et al*., 2020).

7.1. PROTEIN SUBUNIT VACCINES

A protein subunit vaccine formulation often incorporates components of the pathogen that activate the host immune system (Verta *et al*., 2020) in a novel delivery vehicle such as liposome, vrooms, or polymeric nanoparticles (Li *et al*., 2014). Of these liposomes and virosomes are being widely employed in vaccine development against SARS-CoV-2because they not only function as delivery systems for sub unit antigens but also as highly versatile adjuvants(Tandrup Schmidt *et al*.,2016; Liu *et al.,* 2020). A subunit vaccine is the one, which has based on the synthetic peptides or recombinant antigenic proteins, which are necessary for invigorating long-lasting protective and/or therapeutic immune response (Ning *et al.,* 2020). The subunit vaccine, however, exhibits low immunogenicity and requires auxiliary support of an adjuvant to potentiate the vaccine-induced immune responses. The addition of an adjuvant, therefore, helps in overcoming the shortcomings of the protein subunit vaccines (Cao *et al*.,

2018). The S protein of the SARS-CoV-2 is the most suitable antigen to induce the neutralizing antibodies against the pathogen.

These types of vaccines utilize no genetic materials but use whole or fragments of viral Proteins packed in nanoparticles (Pollet *et a*l., 2021). Some vaccines of this type are in preclinical trials utilizing different protein (peptide) subunits (Tan *et al*., 2021). Since these subunits are poorly immunogenic they require adjuvants and repeated administrations (Pollet *et al*., 2021; Arunachalam *et al*., 2021).

7.2 INACTIVATED VIRUS VACCINES

Inactivated vaccine consists of attenuated viral particles or bacterial pathogens that evoke an immune response but not the infection. Since these vaccines do not provide long-lasting immunity, booster doses are often required. Inactivated viral vaccines are formulated by propagating and concentrating large quantities of viral particles and inactivating them via chemical and/or physical methods. Often, ascorbic acid (Madhusudana *et al*., 2004), gamma irradiation (Alsharif and Mulbacher 2010) or relatively high-temperature treatments are employed to inactivate the viral particles. In live vaccines, reverse genetic techniques have being effectively applied to inactivate the exonuclease effects of Protein, and remove the envelope protein in the disease-causing virus (Graham et al., 2013).

The first inactivated COVID-19 vaccine is being developed by the Wuhan Institute of biological Products affiliated with the China National Pharmaceutical Group (Sinopharm). This vaccine is produced by propagating the virus. In phase 1/2 clinical trials, the vaccine generated minimal adverse reactions and has been reported to produce antibodies in all participants (Xia *et al*., 2020).

7.3 ADENOVIRUS VACCINES

Adenoviruses have double-stranded linear DNA enveloped in an icosahedral capsid (Wold and Toth, 2014). Adeno-viruses activate the innate as well as adaptive immunity in mammalian hosts and trigger the release of pro-inflammatory cytokines. These inflammatory cytokines further elevate the immune response by stimulating immune cells such as cytotoxic T lymphocytes,

which recognize and kill virus-infected cells (Zhang *et al*., 2020). Adenoviral vectors have been previously employed against diseases like influenza, Ebola, SARS, influenza, and HIV. Oxford University's, Jenner Institute, CanSino biologics, and Johnson and Johnson have been testing this vector to develop vaccines against COVID-19. Cansino's vaccine, Ad5-nCoV is in phase 2 clinical trials. In addition, Oxford University researchers employ a chimpanzee adenoviral vaccine vector, AZD1222, which is a non-replicating virus. The genetic sequence encoding the S-protein of SARS-CoV-2 is encapsulated in the AZD1222 constructive (Folegatt *et al*., 2020). A single dose of this vaccine has been claimed to generate a strong immune response without causing infection in the vaccinated patient.

7.4. DNA VACCINES

DNA vaccines involve direct administration of a DNA plasmid, which codes for the particular target antigen. This type of vaccine has many potential advantages over conventional vaccines in terms of stimulating both B and T cell responses and has a better safety profile. Since they are devoid of any infectious agents, they could have administered to immune compromised patients (Khan *et al.,* 2013). The DNA plasmid incorporated in the vaccine can be accurately designed using the knowledge of the viral genome. An artificial DNA vaccine directing production of the coronavirus spike (S) protein, the main coronavirus surface antigen presently used in the clinical trial, has been developed to produce a synthetic DNA-based SARS-CoV-2 S protein candidate vaccine based on prior knowledge. (smith *et al*.,2020). The engineered design, INO-4800, leads to vigorous in Vitro expression of the S protein. Efficient antibodies that neutralize SARS-CoV-2 infection inhibit S protein binding to the ACE2 receptor, and the circulation of SARS-CoV-2-targeted antibodies to the lungs was been assessed following immunization (Dham *et al*., 2020). Several DNA vaccine candidates have been reported for SARS-CoV, including the S-, M- and N protein-based vaccines (Wang *et al.*, 2005). Although all of them can generate a certain level of antibody and cell-immune responses, only S protein-based DNA vaccine has been shown to induce protective effect against SARS-CoV infection, probably due to the indispensable role of S protein in receptor binding. (Zhang *et al*., 2013); Yang et al has demonstrated that immunization with DNA encoding full-length S protein, S protein lacking part of cytoplasmic domain, S protein lacking both cytoplasmic and trans membrane domains can all induce neutralizing

antibodies and T-cell immune responses, as well as providing protective effect in mice (Yang *et al*,2004).

Synthetic DNA vaccines accelerate the developmental process of a vaccine as they allow for the quick design of multiple vaccine candidates for preclinical testing, facilitate scalable manufacturing of large quantities of vaccine products, and encounter less regulatory hurdles for clinical translation. Moreover, the synthetic DNA is temperature-stable and has a longer shelf life (Tebas *et al*., 2013). Gene-based vaccines against SARS-CoV-2 are mostly being developed against the S-protein. The vaccine has been expected to trigger the expression of spike antigens in the host, which then induce antibodies capable of inhibiting S-protein recognition by the host receptors. Obviously pharmaceuticals employs DNA-plasmid pGX9501 in their vaccine candidate, which encodes for the S-protein of SARS-CoV-2. Their previous studies have shown that the immunization of animal models with DNA vaccines encoding MERS-CoV S-protein could protect the animal from the disease.

7.5. ANTI-VIRAL DRUGS

Specific effective drugs against SARS-CoV-2 have not yet been discovered and no specific drug has been approved for the treatment of COVID-19. Rapid assessment of the currently available antiviral drugs to be used for COVID-19 patients is therefore crucial in this time of crisis as well as discovering newer drugs (Rodríguez, *et al* 2020). There have been more than 300 clinical trials antiviral drugs going on, and some of them are remdesivir, Favipiravir, Lopinavir, Hydroxychloroquine, Chloroquine, Nitazoxanide, Ivermectin etc. Liu *et al*., 2020. The FDA approved remdesivir (Gilead Sciences, Inc.) for the treatment of COVID-19 requiring hospitalization in patients 12 years of age and older. Remdesivir is an RNA-dependent RNA polymerase inhibitor, which is believed to incorporate itself into SARS-CoV-2 RNA and inhibit its further replication. Remdesivir was also administered to one of the first COVID-19 patients in the United States via intravenous (IV) administration. No adverse effects were reported, and the patient's clinical condition improved even after the supplemental oxygen was discontinued. After the IV administration of 200 mg of remdesivir on the first day and 100 mg on each subsequent day for 9 days, the overall clinical improvement was observed in 36 of 53COVID-19 patients (Grein *et al*., 2020). Lopinavir and ritonavir in an antiretroviral therapy has also has also been

studied in treating patients with COVID-19 but failed to show benefit over standard care (Cao *et al*., 2020).

Table 3 Types of Vaccine for Coronavirus Disease (COVID-19)

Vaccines brand name	Types of vaccine	Approval status by FDA	Country made	Clinical phase trial	Reference
Novavax	Protein subunit	Yes	USA	Phase-3	(https://www.novavax.
Sanofi-GSK	Recombinant protein	No	France-British	Phase-2	Taylor NP (2020).
Moderna	Mrna	Yes	USA	Phase-3	Jackson *et al*., 2020 Anderson *et al*., 2020
Pfizer	Mrna	Yes	USA	Phase-3	Wells and John, 2008
Sinopharm	Inactivated-viruses	Yes	China	Phase-3	(https://valneva.com
AstraZeneca	Adeno-viruses	Yes	British-Swedish	Phase-3	Hollingsworth *et al*., 2020
Johnson and Johnson	Adenoviruses	Yes	USA	Phase-3	Saltman and Beth, 2020
Valneva SE	Inactivated-Whole-viruses	Yes	France	Phase 3	http://www.fdanews.com
Sinovac	inactivated	Yes	China	Phase-1	Lin *et al*., (2007)
Gamaleya research	Adenovirus	Yes		Phase-3	Logunov *et al*., 2020

8. CONCLUSION

The outbreak of a novel virus emerged at the end of December 2019. COVID-19 spread immediately and challenged worldwide medicine, economics, and public health. Today, there are several applications of biotechnology for COVID-19 pandemic in different aspects, such as treatment, diagnosis and prevention. Rapid diagnosis and timely management of this deadly infection by using application of biotechnology are the necessary steps required to eradicate the COVID-19 pandemic. The current standard diagnostic approach still has some limitations regarding a lack of robustness and lengthy procedures. Early detection and isolation of suspected patients can play an essential role in controlling this outbreak. Alternate nucleic acid detection techniques including reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) and CRISPR as well as immunoassay-based devices are currently undergoing through approval phase. In addition, there is an urgent requirement for the development of effective drugs as well as vaccines in order to control this deadly pandemic, which is dangerous to the entire global population.

9. RECOMMENDATIONS

SARS-CoV-2 has recently emerged and has been declared as a pandemic by the World Health Organization. Based on the genomic sequences submitted to NCBI database, the scientific community has analyzed the samples and suggested preventive and therapeutic strategies. Therefore, investigation of genomic diversity in the collected specimens from around the globe needs to be conducted in order to design common, effective therapies and vaccines. In addition, genomic characterization helps us accurately identify the origin and evolution of the virus. The new delta variant SARSCoV-2 ARE spreading more aggressively than previous virus strains because of higher viral load carried by infected individuals. When compared to the earlier virus strains causing covid-19 infectious the delta variant is the most infectious and more transmissible.

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