Republic of Iraq
Ministry of Higher Education
And Scientific Research
University of Diyala
College of Medicine

Corona virus (Covid-19) Vaccines

A project submitted to the council of College of Medicine / University of Diyala in Partial fulfillment of the Requirements for the Degree of bachelor

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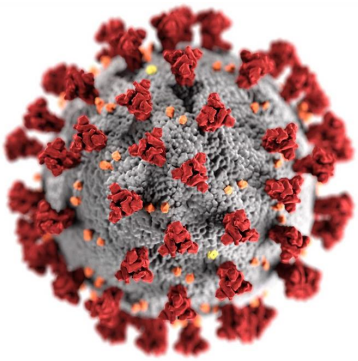

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بسم الله الرحمن الرحيم

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صدق الله العظيم

سورة يوسف آية (76)

الاهداء

الاهداء اولآ الى وجه الله تعالى

في جميع مراحل الحياة يوجد أناس يستحقون منا الشكر والتقدير واولى الناس بالشكر

والدي العزيز الذي جرع الكأس فارغآ ليسقيني قطرة حب

والدتي العزيزة التي وضعتني على طريق الحياة وكان لها الفضل الكبير لنجاحي

والى جميع من وقفوا بجانبي وساعدوني وبالخصوص

الى أصدقائي والى جميع اساتذتي الكرام بوجه عام والى ألدكتور **ناظم غزال نعمان**

المشرف على البحث بوجه خاص الذي كان له دور كبير في اعطائي المعلومات القيمة اهدي لكم بحث تخرجي المتواضع وأتمنى ان تحوز على رضاكم .

ألشكر والتقدير

الحمدلله ألذي هدانا وأعدنا وأمدنا والهمنا الصبر على المشاق ووفقنا لما نحن عليه

فله الحمد والشكر ابتداءاً وانتهاءاً

وارفع كلمة الشكر الى الدكتور **ناظم غزال نعمان**

وفقه الله فقد كانت سندا لي على طول الطريق

والى كل من مد يد العون لي من قريب او بعيد

وقبل ان امضي اقدم اسمى ايات الشكر والامتنان والتقدير والمحبة

الى الذين مهدوا لي طريق العلم والمعرفة

Abstract

The global pandemic of COVID-19 caused by SARS-CoV-2 continues to spread and poses serious threats to public health and economic stability throughout the world. Thus, to protect the global population, developing safe and effective vaccines is mandatory to control the spread of SARS-CoV-2 pandemic. Since genomic sequences of SARS-CoV-2 and SARS-CoV-1 have similarity and use the same receptor (ACE2), it is important to learn from the development of SARS-CoV-1 vaccines for the development of SARS-CoV-2 vaccines. Normally vaccine development takes 10– 15 years but vaccine development against SARS-CoV2 is going on at a very fast pace resulting in almost breakthrough methods of vaccine development by several research institutions. The whole process of vaccine development including clinical trials gets shortened and may be fast tracked to 15– 18 months. Global collaborations and increased research efforts among the scientific community have led to more than 214 candidate vaccines globally. The current review highlights the different approaches and technologies used around the world for the design and development of the vaccines and also focuses on the recent status of the SARS-CoV-2 vaccine candidates under development by various institutions to combat the world threat of COVID-19 pandemic.

List of Contents

Introduction:

On 30 December 2019, three bronchoalveolar lavage samples were collected from a patient with pneumonia of unknown etiology – a surveillance definition established following the SARS outbreak of 2002-2003 – in Wuhan Jinyintan Hospital. Real-time PCR (RT-PCR) assays on these samples were positive for pan-Beta coronavirus. Using Illumina and nanopore sequencing, the whole genome sequences of the virus were acquired. Bioinformatics analyses indicated that the virus had features typical of the coronavirus family and belonged to the Beta coronavirus 2B lineage. Alignment of the full-length genome sequence of the COVID-19 virus and other available genomes of Beta coronavirus showed the closest Relationship was with the bat SARS-like coronavirus strain BatCov RaTG13, identity 96%.

Virus isolation was conducted with various cell lines, such as human airway epithelial cells, Vero E6, and Huh-7. Cytopathic effects (CPE) were observed 96 hours after inoculation. Typical crown-like particles were observed under transmission electron microscope (TEM)

With negative staining. The cellular infectivity of the isolated viruses could be completely neutralized by the sera collected from convalescent patients. Transgenic human ACE2 mice and Rhesus monkey intranasal challenged by this virus isolate induced multifocal pneumonia with interstitial hyperplasia. The COVID-19 virus was subsequently detected

and isolated in the lung and intestinal tissues of the challenged animals. Whole genome sequencing analysis of 104 strains of the COVID-19 virus isolated from patients in different localities with symptom onset between the end of December 2019 and mid-February 2020 showed 99.9% homology, without significant mutation [1,2].

2.1 COVID-19

2.2 Genomic Characteristics of SARS-CoV-2:

Coronaviruses are positive-sense RNA viruses having an extensive and promiscuous wide range of natural hosts and affect multiple systems [3, 4]. Coronaviruses can cause clinical diseases in humans that may extend from the common cold to more severe respiratory diseases like SARS and MERS [5, 6]. The recently emerging SARS-CoV-2 has caused havoc in China, and pandemic situation to the worldwide population, leading to current disease outbreaks that have not been controlled to date through high efforts are being put in to counter this virus. More recently, the WHO announced an official name for this disease as CVID-19.

Coronaviruses possess an unsegmented, single-stranded (ss) positive-sense RNA genome of around 30 kb, enclosed by a 5'-cap and 3'-poly-A tail [7]. The genome of SARSCoV-2 is 29.891 kb long, with a G + C content of 38% [8]. These viruses are encircled with an envelope containing viral nucleocapsid. The nucleocapsids in CoVs are arranged in helical symmetry, which reflects an atypical attribute in positive-sense RNA viruses [7]. The electron micrographs of SARS-CoV-2 revealed a divulging spherical outline with some degree of pleomorphism, virion diameter varying from 60 to 140 nm, and distinct spikes of 9 to 12 nm, giving the virus an appearance of a solar corona [9]. The CoVs genome is arranged linearly as 5′-leader-UTR-replicase-structural genes-(S-E-M-N)-3′ UTR-poly (A) [10]. Accessory genes such as 3a/b, 4a/b, hemagglutinin-esterase gene (HE) are also seen intermingled within the structural genes [7]. The SARS-CoV-2 has also been found to be arranged similarly and encodes several accessory proteins, although it lacks the HE, which is characteristic of some Beta coronaviruses [8]. The positive-sense genome of CoVs serves as mRNA and is translated to polyprotein 1a/1ab (pp 1a/1ab) [11]. A replication-transcription complex (RTC) is formed in double-membrane vesicles (DMVs) by non-structural proteins (NSPS), encoded by the

polyprotein [12]. Subsequently, the RTC synthesizes a nested set of sub genomic RNAs (sgRNAs) via discontinuous transcription [13].

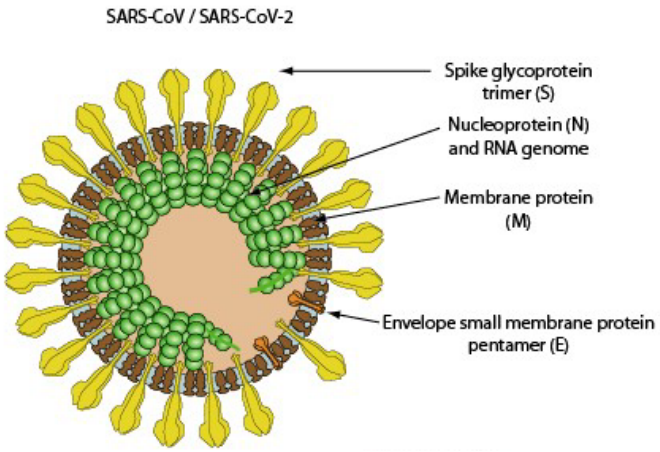


Figure 1. Genomic Characteristics of SARS-CoV-2:

2.3. Transmission

SARS-CoV-2 is readily transmitted between individuals, predominantly via droplet transmission. However, aerosol and faeco–oral routes of transmission might also take place [13]. Individuals are also known to transmit SARS-CoV-2 in the asymptomatic and pre-symptomatic period [14]. During convalescence, patients can shed viral RNA for many weeks,[15,16] and even longer if immunosuppressed [17]. However, there is an unclear association between detectable RNA by quantitative RT-PCR and the ability to culture SARS-CoV-2 in vitro [18]. The closer people interact, and the longer they interact, the more likely they are to transmit COVID-19. Closer distances can involve larger droplets (which fall to the ground) and aerosols, whereas longer distances only involve aerosols [19]. Larger droplets can also turn into aerosols (known as droplet nuclei) through evaporation [20]. The relative importance of the larger droplets and the aerosols is not clear as of November 2020; however, the virus is not known to spread between rooms over long distances such as through air ducts [21]. Airborne transmission is able to particularly occur indoors, in high-risk locations [21] such as restaurants, choirs, gyms, nightclubs, offices, and

religious venues, often when they are crowded or less ventilated.[20] It also occurs in healthcare settings, often when aerosol-generating medical procedures are performed on COVID-19 patients.[20]

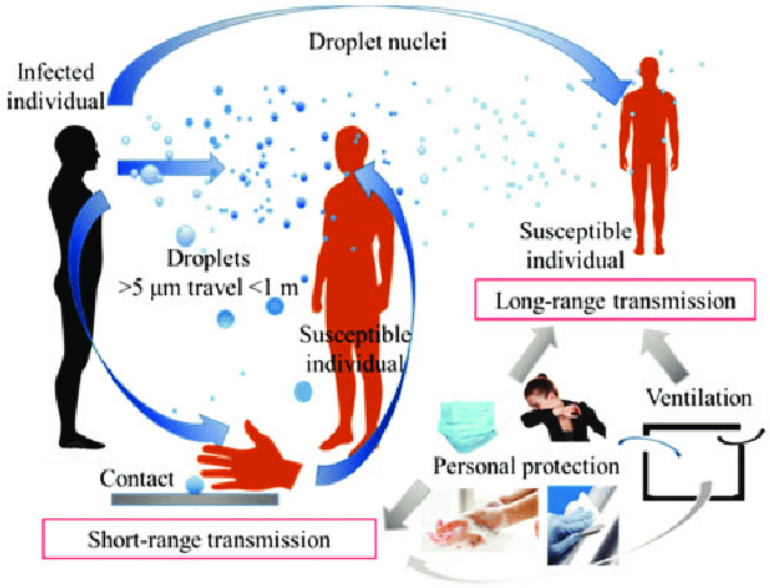


Figure 2. Possible transmission pathways of COVID 19 and personal prevention.

2.4. Epidemiology

The COVID-19 epidemic expanded in early December from Wuhan, China's 7th most populous city, throughout China and was then exported to a growing number of countries. The first confirmed case of COVID-19 outside China was diagnosed on 13th January 2020 in Bangkok (Thailand) [81]. On the 2nd of March 2020, 67 territories outside mainland China had reported 8565 confirmed cases of COVID-19 with 132 deaths, as well as significant community transmission occurring in several countries worldwide, including Iran and Italy and it was declared a global pandemic by the WHO on the 11th of March 2020 [82]. The number of confirmed cases is constantly increasing worldwide and after Asian and European regions, a steep increase in cases is currently (31 March 2020) being observed in low-income countries [83]. It is problematic to

quantify the exact size of this pandemic as it would necessary to count all cases including not only severe and symptomatic cases but also mild ones [84]. Unfortunately, to date, there is not a global and standard response to the pandemic and each country is facing the crisis based on their own possibilities, expertise and hypotheses. Thus, there are different criteria for testing, hospitalization and estimating of cases making it difficult to calculate the number of people affected by epidemic. Based on the data we have so far, the estimated case fatality ratio among medically attended patients is approximately 2%, but, also in this case, a true ratio may not be known for some time [85].

Today, 16 of March 2021, based on the WHO reports, we have globally **120,864,186** Coronavirus Cases**, 2,674,354** Deaths and **97,486,024** Recovered, distributed as follows: Western Pacific Region 103,775 cases and 3649 deaths, European Region 392,757 cases and 29,962 deaths, South East Asia Region 4084 cases and 158 deaths, Eastern Mediterranean Region 46,329 cases and 2813 deaths, Region of the Americas 142,081 cases and 2457 deaths and in the Africa region 3486 cases and 60 deaths [85].

Statistics of Corona virus in the continent of Asia on 16 / March / 2021 **Table 1**, [86]

#	Country, Other	Total Cases	Total Deaths	Total Recovered	Active Cases	Total Tests
	Asia	26,291,565	411,247	24,590,665	1,289,653	
1	India	**11,409,831**	**158,892**	**11,027,543**	**223,396**	**228,280,763**
2	Turkey	**2,894,893**	**29,552**	**2,716,969**	**148,372**	**35,277,116**
3	Iran	**1,763,313**	**61,427**	**1,506,360**	**195,526**	**11,846,528**
4	Indonesia	**1,430,458**	**38,753**	**1,257,663**	**134,042**	**11,740,483**

5	Israel	821,762	6,037	788,685	27,040	12,970,561
6	Iraq	763,085	13,788	690,620	58,677	7,419,942
7	Philippines	631,320	12,848	560,736	57,736	9,360,101
8	Pakistan	609,964	13,595	573,014	23,355	9,565,066
9	Bangladesh	560,887	8,597	514,479	37,811	4,303,994
10	Jordan	486,470	5,428	401,319	79,723	5,223,283
11	Japan	447,906	8,590	426,686	12,630	8,946,247
12	UAE	428,295	1,402	408,085	18,808	34,030,998
13	Lebanon	419,953	5,422	331,506	83,025	3,286,266
14	Saudi Arabia	382,752	6,573	372,926	3,253	14,352,790
15	Malaysia	326,034	1,218	309,612	15,204	6,870,587
16	Georgia	275,685	3,658	268,693	3,334	3,036,115
17	Nepal	275,424	3,014	271,495	915	2,221,512
18	Azerbaijan	241,651	3,298	231,370	6,983	2,726,470
19	Kazakhstan	225,685	2,856	207,371	15,458	8,059,566
20	Palestine	211,602	2,293	187,634	21,675	1,295,750
21	Kuwait	210,855	1,179	195,507	14,169	1,914,579
22	Armenia	179,287	3,277	166,702	9,308	780,085
23	Qatar	170,733	267	158,508	11,958	1,629,260
24	Oman	148,010	1,614	137,028	9,368	1,550,000
25	Myanmar	142,147	3,202	131,739	7,206	2,513,472
26	Bahrain	131,683	485	124,823	6,375	3,321,242
27	S. Korea	96,380	1,678	88,255	6,447	7,126,077
28	China	90,062	4,636	85,244	182	160,000,000

29	Sri Lanka	88,238	532	84,969	2,737	2,236,856
30	Kyrgyzstan	86,990	1,484	83,924	1,582	793,782
31	Uzbekistan	80,743	622	79,371	750	1,377,915
32	Singapore	60,128	30	59,974	124	7,805,264
33	Afghanistan	55,995	2,460	49,481	4,054	322,332
34	Cyprus	39,869	240	2,057	37,572	2,586,414
35	Thailand	27,154	87	26,299	768	1,600,951
36	Maldives	21,666	64	19,245	2,357	581,192
37	Syria	16,556	1,104	11,058	4,394	
38	Tajikistan	13,308	90	13,218	0	
39	Hong Kong	11,330	203	10,786	341	9,296,529
40	Mongolia	4,210	4	3,083	1,123	2,044,869
41	Yemen	2,908	698	1,500	710	17,404
42	Vietnam	2,557	35	2,115	407	2,598,753
43	Cambodia	1,430	1	818	611	555,192
44	Taiwan	990	10	951	29	444,634
45	Bhutan	868	1	866	1	563,755
46	Timor-Leste	203		104	99	24,853
47	Brunei	199	3	185	11	110,176
48	Macao	48		47	1	4,369
49	Laos	48		42	6	121,017
	Total:	26,291,565	411,247	24,590,665	1,289,653	

2.5. Symptoms of COVID19:

Like previous coronaviruses, the novel coronavirus causes respiratory disease, and the symptoms affect respiratory health. According to the Centers for Disease Control and Prevention (CDC), the main symptoms of COVID-19 symptoms can be very mild to severe and include a fever, cough, and shortness of breath. Many people are asymptomatic. Symptoms may appear two to 14 days after exposure. Current information suggests that the virus can cause mild, flu-like symptoms, as well as more severe disease. Most patients seem to have mild disease, and about 20% appear to progress to more severe disease, including pneumonia, respiratory failure, and, in some cases, death [87, 88]

Signs and symptoms of COVID-19 may appear 2 to 14 days after exposure and can include:

- Fever
- Cough
- Shortness of breath or difficulty breathing

Other symptoms can include:

- Tiredness
- Aches
- Runny nose
- Sore throat
- Headache
- Diarrhea
- Vomiting
- Some people have experienced the loss of smell or taste) [89, 90]. Multiple reports have confirmed human-to-human transmission of the COVID-19. When person-to-person spread has occurred with MERS-CoV and SARS-CoV, it is thought to have happened mainly via respiratory droplets produced when an infected person coughs or sneezes, similar to how Influenza and other respiratory pathogens spread. Data has shown that it spreads from person to

person among those in close contact (within about 6 feet, or 2 meters). The virus spreads by respiratory droplets released when someone infected with the virus coughs, sneezes or talks [91, 92].

3.1. Diagnosis Tests

3.2. Rapid Diagnostic Test (RDT)

This is typically a qualitative (positive or negative) lateral flow assay that is small, portable, and can be used at the point of care (POC). These tests may use blood samples from a finger prick, saliva samples, or nasal swab fluids. RDTs are often similar to pregnancy tests, in that the test shows the user-colored lines to indicate positive or negative results. In the context of COVID-19, these tests most frequently test for patient antibodies (IgG and IgM), or viral antigen. In some cases, it can be beneficial to measure the baseline (before infection) of IgG and IgM titers [93,94].

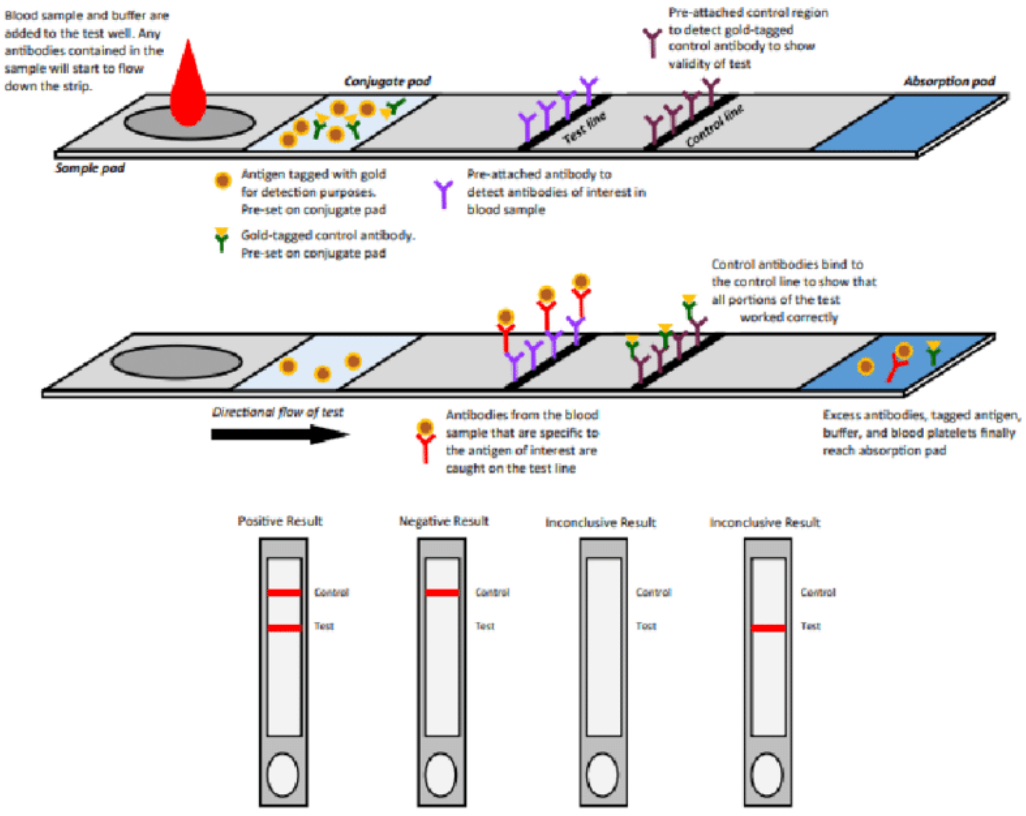


Figure 3. Figure 3: Rapid Diagnostic Test (RDT)

3.3. Enzyme-Linked Immunosorbent Assay (ELISA)

This test can be qualitative or quantitative and is generally a lab-based test. These tests usually use whole blood, plasma, or serum samples from patients. The test relies on a plate that is coated with a viral protein of interest, such as Spike protein. Patient samples are then incubated with the protein, and if the patient has antibodies to the viral protein they bind together. The bound antibody-protein complex can then be detected with another wash of antibodies that produce a color or fluorescent-based readout. In the contest of COVID-19, these tests most frequently test for patient antibodies (IgG and IgM) [95,96].

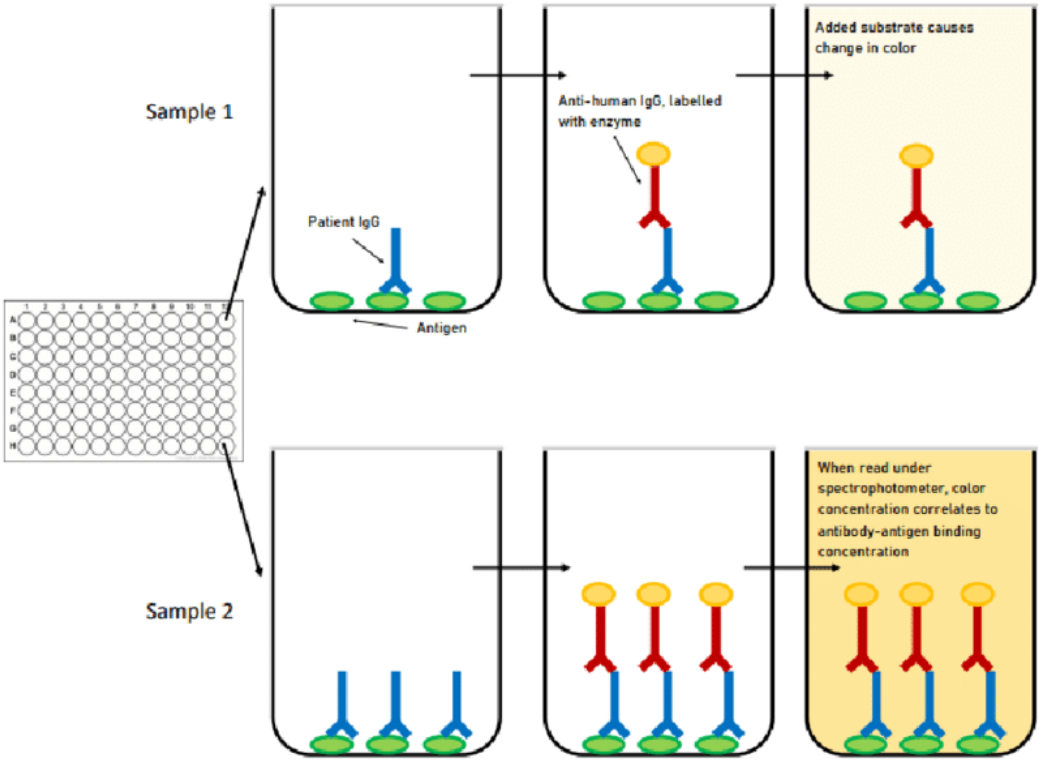


Figure 4: Enzyme-Linked Immunosorbent Assay (ELISA)

3.4. Neutralization Assay

This test relies on patient antibodies to prevent viral infection of cells in a lab setting. Neutralization assays can tell researchers if a patient has antibodies that are active and effective against the virus, even if they have already cleared the infection. These tests require whole blood, serum, or plasma samples from the patient. Neutralization assays depend on cell culture, a lab-based method of culturing cells that allow SARS-CoV-2 growth (like VeroE6 cells). When viruses and cells are grown with decreasing concentrations of patient antibodies, researchers can visualize and quantify how many antibodies in the patient serum are able to block virus replication. This blocking action can happen through the antibody binding to an important cell entry protein on the virus, for example [97,98,99].

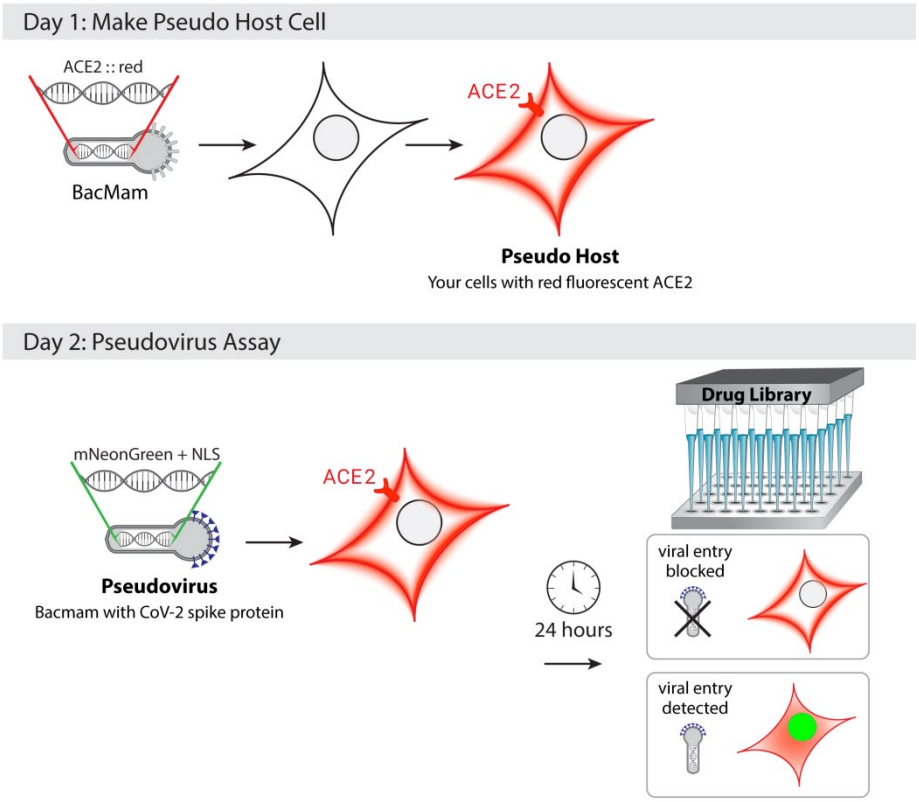


Figure 5: Neutralization Assay

3.5. Chest CT images Chest CT:

is a key component of the diagnostic work-up for patients with suspected infection, and our investigation has shown some imaging findings frequently encountered in affected patients. In the CT scans of lungs, white patches can clearly be seen. Usually, this is a sign of an abnormality that radiologists call ground-glass opacity or a partial filling in of air spaces in the lungs. These kinds of abnormalities are also seen in patients suffering from SARS (Severe Acute Respiratory Syndrome) and MERS (Middle East Respiratory Syndrome)—which are both in the coronavirus family of SARS-CoV-2 [100,101].

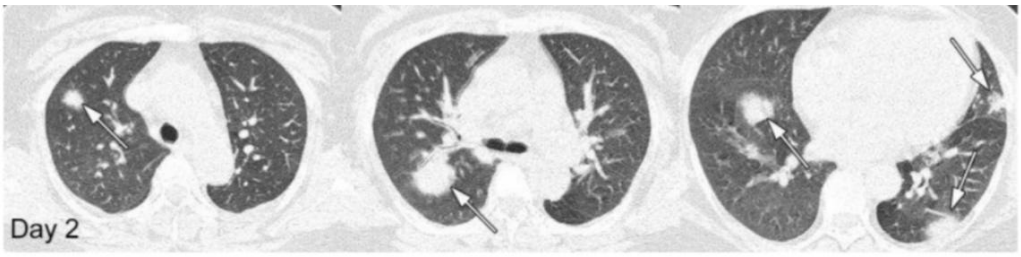


Figure 6: Lungs of a 54-year-old woman who presented with fever on day 2 of symptoms.

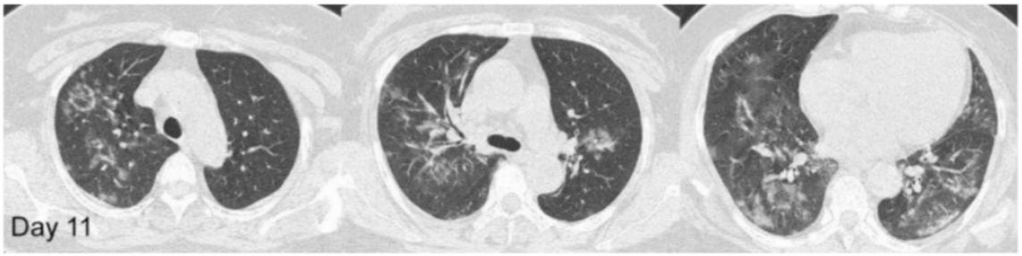


Figure 7: A follow-up CT on day 11, three days after initiation of antiviral treatment, showed significant improvement of the ground-glass opacities

3.6. Thermometer gun

A fever is the body's way of warning you that something is off and can be a sign that your body is trying to fight an illness or infection. For COVID-19, a high fever can be a presenting symptom; however, there are other viruses to consider if fever is the only symptom. Influenza is still prevalent and something to be considered in a patient with a fever. Additionally, some bacterial infections, such as strep throat can cause a fever as well, although additional symptoms are normally present. The CDC considers a person to have a fever

when he or she has a measured temperature of 100.4°F (38°C). A thermometer gun is a device equipped with an infrared sensor that can quickly measure surface temperature without making any contact with a person's skin. In recent years, it has become an important tool for countries scrambling to contain viral outbreaks. It was widely used to try to slow the spread of severe acute respiratory syndrome (SARS) in China in the early 2000s and to curb the Ebola outbreak in West Africa a decade later. But for all of medicine's powerful sensing technology [102,103], the thermometer has ultimately proved to be an ineffective defense mechanism, according to medical officials and experts on infrared devices. Like the surgical masks that have become ubiquitous in China, thermometer guns tend to be unreliable outside carefully controlled health care settings. The thermometers determine temperature by measuring the heat emanating from the surface of a person's body. Often, however, those wielding the tools don't hold them close enough to the subject's forehead, generating unusually low-temperature readings, or hold them too close and get a high reading. The measurements can be imprecise in certain environments, like a dusty roadside, or when someone has taken medication to suppress a fever. It is noticeable that these devices are notoriously not accurate and reliable [104,105].

Figure 8: Thermometer gun

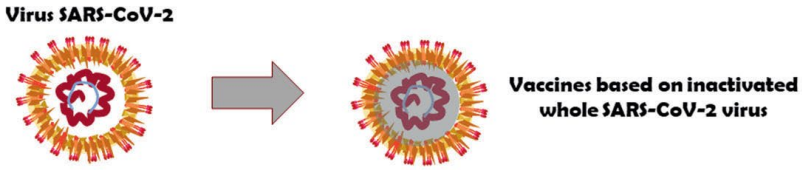
4.1. COVID19 Vaccine

The hope and hype that the media and public at large are placing on having as soon as possible a vaccine that protects against COVID-19 is the result of the great triumphs that vaccines have had and are having in the control of infectious diseases. However, there is a long series of infectious diseases in which vaccines are only partially effective and we have a series of sensational vaccine defeats [22]. Indeed, each disease is an immunological problem in itself: even today, with all the data at one's disposal, it is difficult to predict what kind of vaccine can be truly effective. This difficulty is even greater for COVID-19, a new disease in which ongoing studies in laboratories worldwide are adding new data at a tremendous pace. SARS-CoV2, the coronavirus responsible for COVID-19 is an RNA virus, and these viruses generally have a high mutation rate. Genetic instability has long been considered to represent a challenge to develop effective vaccines against RNA viruses.

In many cases, recovery from a viral disease rests on the combined action of antibodies in the biological fluids that neutralize the viral particles and the killer activity of lymphocytes that track down and kill virus-infected cells. However, there are viral diseases whose healing depends mainly, if not exclusively, on the antibody response and others where the destructive action of the killer lymphocytes is fundamental. What is the case with COVID-19 is not yet clearly defined although several data suggest that the major protective effect is to be attributed to antibodies against the Spike protein and in particular against its receptor-binding domain. Often, healed patients display high titers of SARS-CoV-2 neutralizing antibodies [23]. Data on the role of mucosal immunity and

secretory IgA and IgM are scarce. Furthermore, we cannot yet know how long the protection acquired by recovered patients will last. This point is of interest since often, the duration of the protection after healing somewhat corresponds to the duration of the protection provided by the vaccine.

As of December 2020, just eleven months after the definition of the SARS-CoV-2 genome, there are over 150 official vaccine projects [24, 25]. About fifty of them have already reached human experimentation and a few of these are currently administered to some sectors of the general population. By exploiting different technologies, these anti-SARS-CoV-2 candidate vaccines are targeting the whole SARS-CoV-2, molecules or fragments of molecules expressed on this virus surface. These different candidate vaccines can be grouped based on the technological platform exploited to elicit a protective immune response. However, almost every vaccine project has its peculiarities that make it unique and which could have significant consequences regarding the efficacy or duration of the induced protection or the safety of the vaccine. In **Figs., 10** details of selected vaccine projects that are currently in Phase III trial are shown



Company and country	Vaccine name	Number of doses	Approval and registration
Sinovac Biotech, China	---	2	Ahead of Phase II trial it is offered to essential workers and other high-risk people of the Chinese town of Jiaxing for about 30 €/dose
Beijing Institute of Biological Products and Sinopharm, China	BBIBP-CorV	2	Limited approval for Chinese health care workers and ahead of Phase III trial. This vaccine has been approved by United Arab Emirates on the basis of preliminary data showing that it is 86% effective.
Wuhan Institute of Biological Products, and Sinopharm, China	---	2	Limited approval for Chinese health care workers and soldiers ahead of Phase III trial.
Bharat Biotech, India	COVAXIN	2	The Phase III trial with 26 000 volunteers is expected to close in February 2021.

Figure 9 .Twelve candidate vaccines currently in Phase III trial. COVID-19 vaccines based on the whole inactivated SARSCoV-2.

4.2. Protein Subunit Vaccines

Subunit vaccines may possess antigens with strong immunogenicity that can induce the host immune system. Peptide epitopes isolation is essential for the protein of interest. The epitopes must have the potential to express a strong humoral and T cell immunity against the pathogen that may have a long-lasting effect [26]. Most subunit vaccines require an adjuvant to induce an enhanced immune response. An adjuvant may increase the half-life of the vaccine or may decrease the immunomodulatory cytokine response. So, an adjuvant overcomes the limitation of the protein subunit vaccines. Research for the development of vaccines for SARS-CoV and MERS-CoV give positive inputs [27]. Different forms of S-proteins, including the full-length S protein, S1, and RBD, displayed a varying degree of neutralizing antibody responses and protection from the virus in several studies [28].

4.3. Vaccines Based on Full-Length S Protein

Several institutions utilize the subunit vaccines platform to develop the COVID-19 vaccine, especially when using the S protein as an antigen. The spike (S) protein induces strong immunogenicity, which is the most promising antigen for COVID-19 vaccine development. S protein is the surface protein that mediates virus binding to the host ACE2 receptor for viral fusion and entry. Currently, several vaccine candidates used the S protein to induce an enhanced immune response [30]. The S protein-based vaccines developed against SARS-CoV and MERS-CoV viruses were effective to a large extent. Since SARS-CoV-2 shares a considerable genome sequence and sequence identity of different crucial enzymes with SARS-CoV, the vaccine approaches already built for SARS may facilitate the development of a COVID-19 vaccine [31].

NVX-CoV2373 is under development by Novavax from recombinant full-length S protein with saponin-based Matrix-M1 adjuvant that is Novavax's proprietary nanoparticle technology. Matrix-M induces leukocyte migration into the draining lymph nodes (LN) that increase T cell, B cell, NK, and dendritic cells in draining to LNs. NVX-CoV2373 produces high levels of S protein-specific antibodies that can block ACE-2 human RBD and wild-type SARS-CoV-2 neutralizing antibodies after one dose. NVX-CoV2373 is in Phase 3 clinical trials in the United Kingdom in combination with the seasonal influenza vaccine. Novavax is currently conducting phase 3 clinical trials in the United Kingdom [32,33].

4.4. Protein (RBD-Based) Subunit Vaccine

Subunit vaccines based on recombinant antigenic proteins are essential for expressing long-lasting immune responses. RBD of SARS-CoV-S contains major antigenic epitopes that induce both neutralizing antibodies and T cell responses [33].The immunogenicity of RBD vaccines was better than the full-length S protein. SARS-CoV RBD did not cause immune damage in the animal model, while the full-length S protein could do. It is proposed that there are

super-antigens in the region beyond the RBD of the full-length S protein. Therefore, the RBD-based subunit vaccine may be the ideal and safer alternative to develop a vaccine for Covs [34, 35]. Kentucky Bioprocessing Inc is developing an RBD-based vaccine candidate and undertaking phases I and II clinical trials (NCT04473690). Anhui Zhifei Longcom Biopharmaceutical/Institute of Microbiology, the Chinese Academy of Science has a candidate vaccine that is currently in phase III clinical trial [36].

4.5. Vaccines Based on S2 Subunit

SARS-CoV S2 subunit is responsible for fusion between virus and target cell membrane is proposed to be an effective vaccine candidate against SARS-CoV. Several studies showed that S2 protein induces neutralizing antibodies, although their potency is lower than the RBD-based subunit vaccine. However, Zhang et al reported the immune responses against S2 fragment in BALB/c mice induced a specific cellular immune response [37]. Besides, Zhao et al purified S2-specific IgG from mice immunized with S2 proteins and found that anti-S2 IgGs could abolish the binding between S protein and its cellular receptor(s). Thus, the S2 subunit has a promising potential to be a target to develop a vaccine against divergent virus strains. Currently, several vaccines are under preclinical studies using this platform [38].

4.6. Inactivated Vaccines

Inactivated vaccine platforms have been widely used over the past 70 years. Inactivated vaccines are produced by inactivating the viruses with chemicals, UV light, and heat. Inactivation of the organism makes a safe vaccine, especially for immunocompromised persons. However, these vaccines induce a weaker immune response than live vaccines and need several booster doses. These inactivated vaccines take a longer time to manufacture as the virus needs to be cultured in the lab and then inactivated. Nowadays, seven inactivated

COVID-19 vaccine candidates are in clinical trials, and 12 candidates are in preclinical trials [39,40].

CoronaVac, formerly known as PiCoVacc, is an inactivated vaccine candidate developed by the People's Republic of China's leading vaccine manufacturers, Sinovac Biotech. CoronaVac vaccine is made by proliferating the viruses in cell culture followed by inactivation by formalin with alum adjuvant. Inactivated SARS-CoV-2 viruses possess the RBD within the S protein as immune inducer [40]. CoronaVac was granted an emergency use authorization by Chinese authorities before the initiation of phase III studies. This authorization resulted in 90% of company employees immunized with the vaccine. The phase II clinical trial results by CoronaVac showed that it induced neutralizing antibodies 14 days after vaccination. The neutralizing seroconversion rate of CoronaVac was over 90% in 600 healthy volunteers and can trigger a positive immune response. Currently, CoronaVac is in phase III (NCT04456595) clinical trials [41,42].

New Crown COVID-19 has been developed by Wuhan Institute of Biological Products and Sinopharm as an inactivated whole-virus, alum-adjuvant vaccine. The whole virus cultivated in vitro and infected cells was further inactivated using β-propiolactone and adsorbed to 0.5 mg alum. The phase 1 clinical trial was carried out using three doses (10 μg, 5 μg, and 2.5 μg) of antigen. The results revealed that the vaccine has better safety profiles and strong neutralizing antibody response in all three doses. There were no severe side effects. Phase II clinical trials were undertaken using a 5 μg antigen, and the results displayed New Crown COVID-19 vaccine can effectively generate antibody titer with fewer side effects. A booster dose is essential to produce enough immune response, with (21 days and 28 days) interval between the first doses. The booster dose triggers a better antibody titer as compared to the 14-day intervals. Generally, the New Crown COVID-19 vaccine candidate displayed acceptable safety and a better immunogenic profile, supporting its

assessment in the ongoing phase III trials. A phase III (ChiCTR2000034780) clinical trial began in July 2020 and planned to enroll 21,000 participants. Phase III (ChiCTR2000034780) clinical trials are currently taking place in the United Arab Emirates. Sinopharm gave these candidate vaccines to thousands of people under emergency use conditions approved by the Chinese Government [43,44]. BBIBP-CorV is another inactivated vaccine candidate developed by the Beijing Institute of Biological Products and Sinopharm. In preclinical studies, BBIBP-CorV produced a better immune response in guinea pigs, mice, rats, rabbits, and non-human primates to protect against SARS-CoV-2. In the phase I and II trials, BBIBP-CorV was safe and well-tolerated at all three doses (2 μg, 4 μg, or 8 μg) on days 0 and 28. A robust immune response was observed in 100% of vaccine recipients. The phase III trial of BBIBP-CorV is ongoing in Abu Dhabi, UAE [45].

Bharat Biotech's Covaxin is India's first inactivated vaccine candidate developed by Bharat Biotech, Indian Council of Medical Research, and National Institute of Virology against COVID-19. The phase I and II clinical trials were conducted in 12 hospitals in different cities across the country. The vaccine produced robust immune responses, thereby preventing the SARS-CoV-2 virus. Bharat Biotech is also undertaking a phase III clinical trial with 26,000 participants from across 22 sites in India [46].

4.7. Live-Attenuated Vaccines

Live-attenuated vaccines (LAV) controlled several infections disease outbreaks like yellow fever, mumps, measles, rubella, polio, and chickenpox. The highly attenuated modified vaccinia virus Ankara, recombinant adeno-associated virus, or an attenuated parainfluenza virus encodes recombinant forms of the S protein [47]. Cao et al evaluated the immune protection of a rAAV encoding RBD vaccine in a mouse model by intranasal inoculation, which induced strong mucosal immune responses and provided long-term protection against SARS-CoV infection [48]. Other groups reported that immunization of monkeys via

the respiratory tract with BHPIV3/SARS-S induced the production of SARS-CoV-neutralizing serum antibodies. Weingart et al immunized ferrets with rMVA-S and the immunized ferrets induced neutralizing antibody. After challenging with SARS-CoV, ferrets showed strong inflammatory responses in liver tissue, suggesting that vaccination with rMVA-S enhances hepatitis, which is not observed in the above infection model. It is suggested that we should not only use mice or monkeys as an animal model but also use ferrets as the infection model, because of their sensitivity to SARS-CoV [49]. LAVs associate with the risk of reversion by either mutation- or recombination-driven processes, which cause dangerous outbreaks in unvaccinated populations.
LAV vaccines against SARS-CoV-2 are currently in preclinical development. For example, Indian Immunologicals Limited is currently working together with Griffith University to develop a vaccine using codon deoptimization as a strategy against SARS-CoV-2. The vaccine candidate provides a long-lasting immunity against SARS-CoV-2 following a single vaccination [50]. DelNS1-SARS-CoV2-RBD is an influenza-based vaccine strain with deletion of the virulent NS1 gene. It is attenuated by the deletion of a virulent element and the immune antagonist. It is more immunogenic than the wild-type influenza virus and can be given as a nasal spray. An attenuated version of the influenza virus was modified to encode the RBD domain of SARS-CoV-2 spike protein on its surface [51].

4.8. Nucleic Acid-Based Vaccines (DNA or mRNA) Platform

Nucleic acid-based vaccines offer a cost-effective approach to SARS-CoV-2 vaccine development. In the 1990s Wolf et al reported in vivo the encoded protein expression after administration of encoded nucleic acids (RNA or DNA) into mice [52]. This discovery was the start of the use of nucleic acids encoding antigens as a form of vaccination. While the instability of mRNA limited its use, plasmid DNA emerged as the promising platform even though the first clinical trial was disappointing. Advances in the delivery of vaccines have

spurred new clinical trials to develop vaccines against Covid-19. Currently, several companies are developing nucleic acid-based vaccines [53, 54].

4.9. mRNA Vaccine

The mRNA-based vaccine is an emerging, non-infectious, and non-integrating platform with less risk of insertional mutagenesis. It is the most promising alternative due to its cost and safety profile in animal studies. The immunogenicity of the mRNA can be minimized, and alterations can increase the stability of these vaccines. Furthermore, the anti-vector immunity can be removed as the mRNA is the minimally immunogenic genetic vector, allowing repeated use of the vaccine. This platform has empowered the rapid vaccine development program due to its flexibility and ability to mimic the antigen structure and expression as seen in the course of natural infection. Currently, six mRNA-based vaccine candidates are in the clinical trials, and 19 vaccine candidates are in the preclinical trial for COVID-19 [55, 56].

mRNA-1273 is an mRNA vaccine with a synthetic viral mRNA, which encodes the full-length spike protein (S) of SARS-CoV-2 and mimics the natural infection. The host body recognizes the synthetic viral mRNA and translates the viral protein shown in. mRNA is not stable, but through chemical modifications, mRNA is stabilized and packaged into an injectable form using a liquid nanoparticle [55, 57]. The mRNA-1273 is a lipid nanoparticle (LNP)-encapsulated mRNA-based vaccine that encodes a stable form of the spike protein of SARS-CoV-2. The encapsulated mRNA-1273 then travels to the immune cells (lymph nodes) and instructs them to make copies of the spike protein on their surface as if SARS-CoV-2 infects them. Other immune cells learn about the spike protein and prepare themselves for the future response to SARS-CoV-2. mRNA-1273 evoked neutralizing antibody titer levels observed in convalescent sera within their initial 45 participants. It is also generally safe and provided complete protection in a mouse challenge model. Moderna has collaborated with Catalent since June 25, 2020 to perform large-scale,

commercial fill-finish manufacturing of Moderna's mRNA-1273 COVID-19 vaccine at Catalent's biologics facility in Indiana, US. Besides, it is relatively safe as it is neither an inactivated pathogen nor the subunits of the live pathogen. mRNA used in vaccination is safe to use since it cannot become part of a person's chromosomes [58,59]. Most adverse events were mild or moderate, with Grade 3 (severe) in 2%, typically pain at the injection site and fatigue, achiness, joint pain, headache, and redness at the injection site after the second dose. Moderna reported that the mRNA-1273 vaccine candidate passed its most important test, the 30,000+ participant phase III clinical trial (NCT04470427) by showing 94.5% effectiveness in preventing COVID-19. The FDA advisory panel voted to recommend the approval of the mRNA-1273 vaccine for emergency use [60].

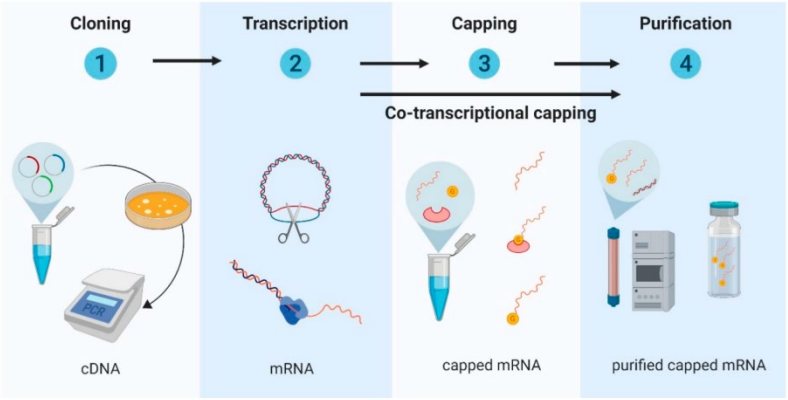


Figure 10: RNA vaccine platforms.

BNT162b1/BNT162b2 vaccines are codon-optimized mRNA vaccines that encode SARS-CoV-2 RBD and full-length spike respectively. The mRNA is encapsulated in 80 nm ionizable cationic lipid nanoparticles that increase its delivery. The phase II and III clinical trials showed the two vaccine candidates elicited similar dose-dependent SARS−CoV−2−neutralizing geometric mean titers (GMTs), comparable to the GMT of a panel of SARS−CoV−2 convalescent sera. BNT162b2 showed less systemic reactogenicity, particularly in older adults [61].

The ongoing phase 3 clinical trial of BNT162b2, has enrolled more than 44,000 participants, from 150 sites in the US, Germany, Turkey, South Africa, Brazil, and Argentina. Most participants have received their second dose. The phase III trial BNT162b2 was 95% effective 28 days after the first dose. FDA granted Pfizer and BioNTech an emergency use authorization (EUA) of the BNT162b2 COVID-19 vaccine [62].

THE LUNAR-COV19 (ARCT-021) vaccine contains a lipid-mediated delivery system called Lipid-enabled and Unlocked Nucleomonomer Agent-modified RNA (LUNAR). After administration, the vaccine enters the host's cells, where the mRNA translates into proteins. These proteins are from the SARS-CoV-2 virus, and thus the host can induce an immune response against them and fight off an infection from the native virus. It is developed by Arcturus Therapeutics on an RNA-based vaccine platform and enters phase I and II clinical trials in Singapore [63].

4.10. DNA Vaccines

The most revolutionary approach to vaccination is the introduction of the DNA vaccine, which encodes antigen, and induces the adaptive immune response. The transfected cells express the transgene that provides a steady supply of the transgene-specific proteins, which is similar to the live virus [64]. DNA vaccines targeting S, M, and N proteins induce humoral as well as cellular immune response. Although the DNA vaccine platform is temperature stable and rapidly manufactured, their efficacy and immunogenicity in persons are not yet proven. DNA vaccine administration, vector mutations, and genome integration into the host gene are remaining issues. DNA vaccine platform was started in 1993 with promising results against influenza viruses, but the same results cannot be translated to humans yet [65].

INO-4800 is a DNA vaccine candidate developed by Inovio Pharmaceuticals. It was designed to optimize the S protein sequence of the SARS-CoV-2 virus. The presence of humoral and T cell response in the preclinical trials suggest

that *INO-4800* can produce an effective immune response. The vaccine has entered phase I and II (NCT04336410) clinical trials. The clinical trial will evaluate the immunological profile, safety, and tolerability of the vaccine candidate [66].

4.11. Replicating and Non-Replicating Viral Vectors Vaccines

Viral vectors vaccine is a promising prophylactic solution against a pathogen. These vaccines are specific in delivering the genes to the target cells, highly efficient in the gene transduction, and induce the immune response. Viral vectors vaccine provides prolonged time and enhanced antigenic protein expression and, therefore, has better prophylactic use as these vaccines trigger the cytotoxic T cells, which leads to the removal of the virus-infected cells. Viral vectors are grouped into replicating (measles virus and vesicular stomatitis virus) or non-replicating vectors (Adenoviruses (Ad) and poxviruses). Several vaccine developments rely on non-replicating Ads vector. These vectors are relatively safe, physically, and genetically stable, and do not integrate into the host genome. Ads vector can infect DCs and dividing or non-dividing cells. However, Ad vectors need high doses to induce the host immune response [67,68].

AZD1222 (ChAdOx1 nCoV-19): is a SARS-CoV-2 vaccine candidate that uses a non-replicating chimpanzee adenovirus as a vector (ChAdOx1) and is modified to induce the S protein from SARS-CoV-2. AZD1222 is under development by the University of Oxford and AstraZeneca. This adenovirus is genetically modified so that it does not replicate in humans [63]. After vaccination, cells induce spike protein and the critical immune system to produce neutralizing antibodies that bind to spike glycoprotein and attack SARS-Cov-2 virus. Besides, the vaccine induces T-cells, which can attack the host cells if they get infected by the SARS-CoV-2 virus. Phase I and II studies show that the candidate AZD1222 vaccine given at a dose of 5×10^{10} viral particles was safe and tolerated, however higher reactogenicity was displayed

than the control vaccine. The reactogenicity decreased with 1 g paracetamol for the first 24 hours after vaccination. Generally, the two doses were enough to induce a potent immune response in all the participants and had no severe adverse reactions. Phase III trials are currently ongoing in several countries [67,69].

On June 29, 2020, the People's Republic of China's military received permission to use the Ad5-nCoV vaccine (Adenovirus vaccine) candidate developed by the People's Republic of China's CanSino Biologics Inc. as it was proved safe and efficient in the clinical trials (NCT04341389). Ad5-nCoV uses replication-defective human Ad5 as a vector and is modified to express the S protein. This vector was used for the Ebola vaccine (Ad5-EBO) development. Ad5-nCoV vaccine candidate developed by the People's Republic of China's CanSino Biologics Inc. as it was proved safe and efficient in the clinical trials (NCT04341389). The phase I clinical trials showed four-fold in RBD and S protein-specific neutralizing antibodies and specific T cell responses. However, the pre-existing anti-Ad5 immunity may antagonize both the antibody and the T cell responses before an immune response to the S protein develops [70,71].

Coroflu (M2SR) is a unique influenza virus. It lacks a gene called M2 that limits the influenza virus to undergo only a single replication in cells. M2SR is modified by the insertion of the spike protein gene sequence of the SARS-CoV-2. It can enter into the host cell, thereby inducing an immune response against the SARS-CoV-2. It is administered intra-nasally, mimicking the natural route of viral infection. The immune response is higher as compared to the intramuscular injections. It is in the preclinical phase [72].

LV-SMENP-DC vaccine: Dendritic cells are antigen-presenting cells which induce immune response via antigen presentation. Dendritic cells exist in the periphery and are active at engulfing and presenting exogenous antigens. LV-SMENP-DC vaccine is developed by modifying dendritic cells and T cell activation by the lentiviral vector to encode COVID-19 antigen. The

subcutaneous inoculations of the vaccine present the cytotoxic T cells and generate the immune response. LV-SMENP is currently undergoing a phase I and II multicenter trial (NCT04276896) in healthy volunteers [73,74].

On August 11, 2020, Russia approved the "Sputnik V" anti-SARS-CoV-2 vaccine developed by Moscow's Gamaleya Institute. It is not clear if the completion of all phases of clinical trials were carried out. Phase III clinical trial for its effectiveness would be done after regulatory approval. Sputnik V uses a non-replicating viral vector "adenovirus" containing gene-specific spike protein like other vaccine candidates. The difference with other candidate vaccines is that Sputnik V uses two adenovirus vectors: Ad26 and Ad5, instead of a single serotype. In the first dose, Ad26 is given and, the second dose of Ad5 is administered after 21 days. This strategy has an advantage as, after the first dose, antibodies are produced against the Ad26 serotype. The second dose is of Ad5 serotype; therefore, the body is stimulated to produce an enhanced immune response. Phase I and II trial results of Sputnik V have shown antibody production in all the trial participants, though the sample size was small, and further studies will be carried out in the elderly [75,76]. After the announcement of Sputnik-V, the Russian government received preliminary orders for greater than 1 billion doses of Sputnik V vaccine from 20 countries. Now, Russia is manufacturing more than 500 million doses of Sputnik V vaccine [76].

4.12. Virus-Like Particle Vaccines

VLPs mimic the conformation of native viruses. VLPs contain almost all viral proteins, but lack a viral genome that causes disease transmission and non-structural proteins. Due to a lack of genetic material, VLPs cannot replicate in the host but can express both the cellular and humoral immune responses. VLP can be produced using a combination of structural proteins from various types of viruses and plants. Currently, several VLP-based vaccines are available such as HBV and HPV vaccines for prophylactic use [77].

Plants are an ideal platform for oral vaccine production. The genome for viral proteins is delivered to the plant using *Agrobacterium bacteria*. After infection, the gene of interest integrates into the plant genome and produce virus-like particles. Plant-based vaccines produced using *Agrobacterium* and *Nicotiana benthamiana* against influenza A viruses (A/H1N1, A/H3N2), and avian influenza H5 (AIV) were effective, safe, and well-tolerated. This vaccine platform was displayed as a promising source of vaccine for Lyme disease, Newcastle disease, bovine viral diarrhea virus, and recombinant colicin M. Several studies proposed different recombinant VLP vaccines against SARS-CoV-2 viruses [78.79].

Currently, several VLP vaccines under development are RBD SARS-CoV-2 HBsAg VLP vaccine in phase I and II (ACTRN12620000817943) developed by SpyBiotech and Serum Institute of India, and CoVLP is in phase II/III (NCT04636697) developed by Medicago biopharmaceutical in Canada. The Medicago Inc. vaccine was developed by using its proprietary plant-based technology using tobacco plants to produce virus-like particles. The RBD-HBsAg-VLPs-Covid vaccine based on the RBD domain of SARS-CoV-2 conjugated to the hepatitis B surface antigen (HBsAg) virus-like particles [80].

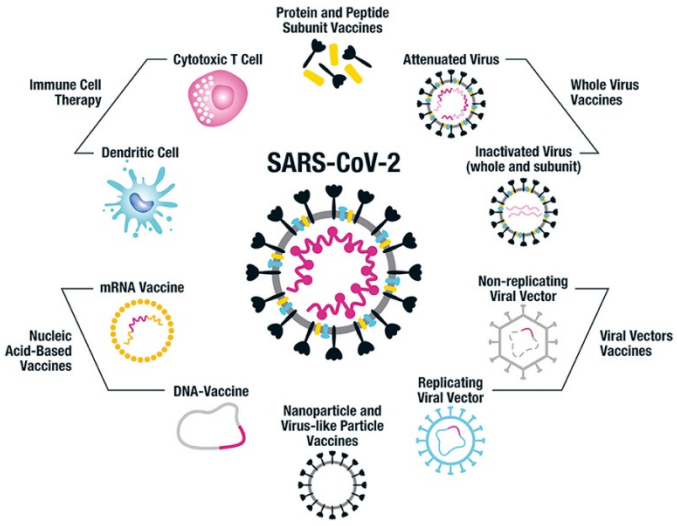


Figure 11. COVID19 vaccination

4.13. The best vaccine for Corona virus:

The Pfizer–BioNTech COVID-19 vaccine it's the best vaccine and it's used by many countries, it sold under the brand name Comirnaty, is an mRNA based COVID-19 vaccine. The German company Biotech is the initial developer of the vaccine, and partnered with American company Pfizer for support with the clinical trials, logistics and manufacturing, It is given by intramuscular injection. It is composed of nucleoside-modified mRNA (modRNA) encoding a mutated form of the spike protein of SARS-CoV-2, which is encapsulated in lipid nanoparticles. The vaccination requires two doses given three weeks apart. It is one of the two RNA vaccines deployed against COVID-19 in 2020, the other being the Modern COVID-19 vaccine. Clinical trials began in April 2020; by November the vaccine entered phase III clinical trials, based on more than 40,000 people. An interim analysis of study data showed a potential efficacy of over 90% in preventing infection within seven days of a second dose. The most common side effects include mild to moderate pain at the injection site, fatigue, and headache. Reports of serious side effects, such as allergic reactions, have been very rare,[a] and no long-term complications have been reported. Monitoring of the primary outcomes from the trials will continue until August 2021, while monitoring of the secondary outcomes will continue until January 2023[106] .

How some of the Covid-19 vaccines compare

Company	Type	Doses	How effective*	Storage
Oxford Uni-AstraZeneca	Viral vector (genetically modified virus)	x2	62-90%	Regular fridge temperature
Moderna	RNA (part of virus genetic code)	x2	95%	-20C up to 6 months
Pfizer-BioNTech	RNA	x2	95%	-70C
Gamaleya (Sputnik V)	Viral vector	x2	92%	Regular fridge temperature

*preliminary phase three results, not yet peer-reviewed

Figure 12.COVID-19 Vaccination

Figure 13.A vial of the Pfizer–BioNTech COVID-19 vaccine

Conclusion:

Assessment of the efficacy of a vaccine is complex for many diseases but particularly so in the case of SARS-CoV-2, where the fundamental understanding of the pathogen is evolving. Multiple vaccines are being tested worldwide in early-phase studies and some vaccine candidates are already in phase 3 studies assessing efficacy.4 It is probable that there will not be a single vaccine winner; diverse platforms and technologies can offer different strengths and be relevant in distinct epidemiological contexts. Additionally, there will probably be insufficient supply, at least initially, of a single vaccine. However, collaboration and standardized approaches for assessing different efficacy endpoints will be important to allow meaningful comparison and ensure that the most effective candidates are deployed. Following deployment, well supported pharmacovigilance studies should be established to ensure the ongoing evaluation of vaccine safety.

Capacity to measure vaccine efficacy in field studies is reliant on ongoing SARS-CoV-2 transmission, which is rightly at odds with public health interventions. In the absence of a surrogate of protection, CHIM trials might provide the only means of rapidly assessing vaccine efficacy; however, the relationship between efficacy data from CHIM studies in young individuals and population-level protection is unclear. CHIM studies might help to identify a surrogate of protection. It is probable that any evidence for efficacy against severe disease and mortality in populations that are at risk will only be garnered post licensure via large epidemiological studies.

Finally, the development of SARS-CoV-2 vaccines is under great political and media scrutiny. In keeping with the development of any novel medical intervention, but particularly so in this context, it is imperative that efficacy outcomes for a SARS-CoV-2 vaccine are critically appraised with scientific rigour to understand their generalizability and clinical significance.

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