Development of New Screening Methods for SARS-CoV-2 and its Associated Developing Variations

Deepika Kumari[#], Anil Kumar Mavi[%], Jyoti Chaudhary[#], Manoj Kumar^{@,} Rajesh Kumar Gupta[^], Umesh Kumar^{§*}

*Department of Biotechnology, School of Engineering & Technology, Noida International University, Greater Noida, Uttar Pradesh -203 201, India

[%]Department of Botany & Life Sciences, Sri Aurobindo College, University of Delhi, Delhi - 110 017, India

[®]Department of Pulmonary Medicine, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi - 110 007, India

[^]Department of Applied Chemistry, School of Vocational Studies, and Applied Sciences, Gautam Buddha University, Greater

Noida, Uttar Pradesh - 201 312, Uttar Pradesh, India

[§]Department of Biosciences, Institute of Management Studies Ghaziabad (University Courses Campus), NH09, Adhyatmik

Nagar, Ghaziabad, Uttar Pradesh - 201 015, India

*E-mail: umeshkumar82@gmail.com

ABSTRACT

As new and changing SARS-CoV-2 variants are discovered, there is an increasing demand for more adaptable diagnostic tools capable of detecting SARS-CoV-2 infections. The wide range of symptoms experienced by infected individuals and unexpected variants make it more challenging than ever to create quick and accurate diagnostic tools. Pharmaceutical treatments and vaccinations are continually designed to strengthen the immune function and successfully combat SARS-CoV-2 and its variations. The discovery of new SARS-CoV-2 mutations and variants, along with the advancement of diagnostic methods that make it possible to identify them, have brought up a number of urgent issues that are covered in this review from a completely fresh perspective. Additionally, we go over the creation, composition, operating principles, benefits, and downsides of some of the most popular vaccinations and therapeutic medications, as well as the ensuing immunological influence.

Keywords: SARS-CoV-2; Vaccine; Pharmaceutical therapy; Challenges

1. INTRODUCTION

SARS is the first disease of the twenty-first century with the potential for a global outbreak. Around the middle of November 2002, the virus was first discovered in China's Guangdong Province¹. In December 2019, clinical indicators of a cluster of pneumonia patients with viral pneumonia initially surfaced in Wuhan, Hubei, China. Samples from the lower respiratory tract were extensively sequenced to search for a new corona virus¹. Severe Acute Respiratory Syndrome Corona virus 2 (SAR-CoV-2) was officially renamed Corona virus 2019 (COVID-19) on February 11, 2020 by WHO. Respiratory failure, a high body temperature, coughing, fatigue, pneumonia, and muscle pain are symptoms of the sickness².

Corona viruses (CoVs) are found in the Coron avirdiae family, a subfamily of the Corona virinae family, and the order Nido virales. These viruses are distinguished by their enclosed, positive sense, singlestranded RNA structure and average size of 80-220 nm. The distinctive shape found under electron microscopy resembles a coronal arrangement (Fig. 1). Developing acquired immunity against the pathogen referred to as the

Received : 9 June 2023, Revised : 19 September 2023 Accepted : 30 October 2023, Online published : 21 August 2024 severe acute respiratory syndrome corona virus (SARS-CoV-2) is the main objective of COVID-19 immunization. The assessment of the current SARS-CoV-2's structure and pathogenic processes has been considerably supported by knowledge gained from previous outbreaks of corona viruses with characteristics similar to SARS and the Middle East Respiratory Disease (MERS).

There are no known mechanisms or modes of transmission for the SARS and MERS viruses. SARS-CoV and MERS-CoV spread predominantly by nosocomial transmission. During each pandemic, hospitals had a 43.5-100 % association with MERS patients, equivalent to SARS patients³.



Figure 1. Schematic of structure of SARS-CoV-2.

The Target Product Profile states vaccinations desired and minimally desired features⁴. These critical characteristics of vaccine include: (1) Vaccines have a very favorable benefit-risk profile in terms of protection and immunogenicity, with only slight, transient adverse events; (2) Vaccines generate at least 70 % effectiveness when evaluated for populationlevel efficiency against illness, serious disease, and "shedding/transmission," with similar outcomes in older people; (3) Vaccinations are designed to address all age groups within a given population; (4) One dose of the vaccine can be administered, and annual or less regular booster shots can ensure immunity for a long time; (5) Specific protection lasts for at least a year; (6) In order to improve epidemic control, nonparenteral delivery techniques are being researched; (7) Thermo stable vaccines are designed to tolerate higher temperatures, allowing for increased temperature stability and (8) Multi-dose vaccine generation can be scaled up at a price that makes them generally accessible, even in nations with middle- or lowincome levels.

In this review, we give a fresh perspective on the issues that have developed as a result of the emergence of novel SARS-CoV-2 mutations and variations, as well as the advancement of diagnostic technologies that allow them to be identified. We also discuss how some of the most popular vaccines and treatments were created, how they were assembled, how they worked, what benefits and drawbacks they had, and how this affected how the immune system responded.

2. DETECTION OF SARS-COV-2

As more SARS-CoV-2 variations are found and created, the demand for adaptable diagnostic tools for identifying SARS-CoV-2 infection grows. The incidence of mutations, on the other hand, and the symptomatology reported by affected patients, make it more difficult to develop quick and accurate diagnostic techniques. Methods for detecting SARS-CoV-2 primarily rely on certain viral proteins, nucleic acids, or antiviral antibodies¹.

2.1 Nucleic Acid

Genetic material detection is currently a regularly used method for the clinical detection of SARS-CoV-2 infection. According to research, nose swab samples are the most reliable way to conduct tests because of their high sensitivity rate of 97 %, which is higher than that of alternative sources including oral swabs (85 %), nasopharyngeal swabs (86 %), and oropharyngeal swabs (68 %) RT-PCR detects particular viral RNA sequences from the Envelope, Nucleocapsid, Spike, and ORF1ab domains⁵. The accuracy of this method can be endangered by viral changes, resulting in unexpected test results and incorrectly negative outcomes¹². VOC-specific primers for real-time PCR detection are being developed. In this study, two innovative PCR-based techniques for identifying and distinguishing VOCs from common SARS-CoV-2 strains were examined. LAMP, or loop-mediated isothermal amplification, was initially created as a fast, dependable, and costeffective replacement for conventional RT-PCR-based diagnostics¹. This approach shows accuracy as it uses 6–8 primer sequences to pinpoint eight distinct target locations. The review is commended for emphasizing the SARS-CoV-2 pathogen's E and N genomic transcripts. The sensitivity and specificity of the CRISPR-Cas13 assays are both greater than 95 %⁶.

SARS-CoV-2 can be identified using gene microarray technology, namely the 60-mer DNA microarray¹. The probes affixed to the surface of the microarray were used to examine the entire viral DNA. The RNA extracted from SARS patients' pharyngeal biopsies and gargle fluid samples was used to generate CDNA by reverse transcription. The CDNA was then broken up and visualized using restriction display technology. Before developing PCR-based hybridization, Cy5-tagged universal primers were employed to label the fragmented DNA. Despite the absence of signals in the negative and blank controls, the data show that microarray technology can differentiate the SARS coronavirus⁷.

Next-generation sequencing (NGS) technology can be utilized to detect and comprehend the SARS-CoV-2 virus. Despite the dependability and accuracy of NGS systems, their implementation is frequently hampered by a lack of funding and expertise⁸.

2.2 Antibodies and Immunoassays

Various antigen-based immunoassays such as immunefluorescence tests, lateral flow tests, chemiluminescent immunoassays, and ELISA can be employed to accurately detect SARS-CoV-2 infections. Saliva, nasal swabs, and nasopharyngeal swabs are only a few clinical samples for which commonly accessible assays have proven beneficial⁹. The primary goal of SARS-CoV-2 detection is the identification of two critical proteins, specifically the S and N proteins. The efficiency of these diagnostic procedures is greatly impacted by factors such as the severity of the sickness and the amount of virus present. The current research looks into antigen-based immunoassays' detection rate using modern sensing and biosensor technology⁸.

2.3 Detection Utilising Antibodies

The early detection of SARS-CoV-2 patients using antibody-based detection techniques is anticipated to be less successful than nucleic acid and antigen-based detection strategies. This is because antibody reactions commonly occur two weeks following infection when virus nucleic acid and antigen levels are at their lowest¹⁰.

Antibodies made against the SARS-CoV-2 viral antigen are precisely detected using a variety of binding techniques, including immuno fluorescence analysis,imm unochromatography,chemiluminescence tests, and ELISA. These devices have the capacity to produce antibodies that bind to viral illnesses' S and N proteins with excellent specificity. Blood's immunoglobulin M/IgG ratio can be measured using a variety of commercially available tests⁹. Humoral immunological reactions to SARS-CoV-2 can be found using straightforward blotting techniques. Typically, they are capillary-based, highspeed automated methods for identifying human IgG reactions against the five main SARS antigens in either plasma or serum samples¹.

2.4 Nanotechnology-Based Methods

The diagnosis process is fairly simple, and the test is successful when the hue changes in the presence of plasmonic gold nanoparticles. For this test, DNA sequencing or other complex scientific procedures are not required. Using this method, virus RNA can be found on the first day of transmission¹¹.

RNA is extracted within 10 minutes of collecting saliva from the patient's lips or nasal mucosa. The assay looks for specific proteins using gold nano particles. The liquid solution turns blue when the biosensor's gold nano particles are connected to the virus's DNA sequence (Fig. 2). The accuracy of the COVID-19 test is critical to understanding infection. In other words, whether or not the pathogen is present, the result will not be wrongly negative or positive. According to various currently available diagnostic procedures, the illness is difficult to detect for several days after infection¹². As a result, many of these studies' adverse conclusions must be revised. The authors conclude that because it does not require laboratory setup or specialist employees to administer and evaluate, this test is substantially less expensive to create and utilize than laboratory tests. This procedure complies with FDA regulations. This strategy is applicable in a variety of settings, including schools, nursing homes, workplaces, and college campuses¹¹.

3. VACCINES FOR SARS-COV-2 (COVID-19)

3.1 mRNA Vaccines

mRNA vaccines were discovered to be efficient against a variety of viral infections. Lipid nanoparticles in these vaccines maintain the perfusion and protect the S protein-encoding mRNA when it enters the intracellular space. The host generates the S protein through mRNA, which results in a focused immune response. Studies show that mRNA-based vaccines like Moderna's mRNA-1273 and Pfizer-BioNTech's BNT162b2 are more than 90 % efficient¹. This technique has several advantages, including the ability to generate TH1 and TH2 responses and the ability to create vaccines quickly¹³. Both vaccinations are administered in two doses through intramuscular injection, but the timing of the second dosage varies between BNT162b2 and mRNA-1273. The second dose of BNT162b2 is given 21 days after the first, whereas the second dose of mRNA-1273 is given 28 days after the first.

In phase III clinical trials, BNT162b2 and mRNA-1273 vaccination recipients displayed a protective effectiveness of more than 90 % in people with no known medical history. The BNT162b2 immunization, according to Polack et al., prevents COVID-19 in people who are 16 years old and older with a 95 % efficacy rate and few serious adverse effects¹⁴.

Thompson *et al.* claim that administering two doses of mRNA vaccine results in a full immunization efficacy of 90 %. The mRNA-1273 COVID-19 vaccine, on the other hand, is only approved for adults 18 and older, and administration is only permitted 14 days after the second dosage. According to studies, the COVID-19 variants seen in South Africa (B.1.1.7) and the United Kingdom (B.1.1.7) are well protected (95 %) against the BNT162b2 vaccination (B.1.351)¹⁵.



Figure 2. Well formulated AuNPs mediating SARS-CoV-2 selected.

Vaccine's name	Manufactured by	Vaccine category	Antigen	Dose	Dosage	Storage condition	Effectiveness against severe COVID-19	Current approvals
mRNA-1273	Moderna (US)	mRNA vaccine	complete spike protein with proline employed as a replacement	100µg	2 dosage 28 days apart	-25° C to -15°Cf for 30 days; RT ≤12h	100% at 14 days following the second dose	EUA: The US,EU, Canada and UK
BNT162b2	Pfizer-BioNTech (US)	mRNA Vaccine	Proline substituted full-length spike protein	30 µg	2 Dose 21 Days apart	RT = 2h; -For 5 days, temperatures ranged from 80° C to -60° C.	88.9% after 1 dose	EUA: The US, EU, Canada and the UK
CVnCoV	Cure Vac/ GlaxoSmithKline (Germany)	mRNA Vaccine	Perfusion was used to stabilize the full-length S protein of SARS-CoV-2.	12 µg	28 days between each dose of two	3 months at 2-8°C; RT for 24 hours	Unknown	
NVX- CoV2373	Novavax.inc (US)	Protein subunit Vaccine	S protein perfusion, full-length recombinant	Matrix-M adjuvant (50 g) and protein (5 g).	2 doses	2-8°C for 6 months	Unknown	EUA application planned
Ad26.CoV2	Janssen/Johnson& Johnson (US)	Viral vector vaccine	a full-length, stable, and replication-impaired recombinant human adenovirus serotype 26 vector encoding the SARS-CoV-2 protein.	5.1 billion virus particles	1 dose	3 months at -20°C and 2-8°C	85% After 28 days; 100% after 49 days	EUA: the USA, EU and Canada
ChAdO×1 (AZS1222)	AstraZeneca/Oxford (UK)	Viral vector vaccine	SARS-CoV-2 S protein in a chimpanzee vector with poor replication	A normal dose of virus particles is 5 x 10 ₁₀	(Intervals >12 weeks investigated) 2 doses were administered 28 days apart	2-8° C for 6 months	100% after the first dose, 21 days	EUA: WHO/ Covax, the UK, India and Mexico
Gam- COVID-Vac (Sputnik-V)	National Center for Microbiology and Epidemiology in Gamaleya	Viral vector Vaccine	full-length adenoviral vector Glycoprotein S of SARS-CoV-2	10 ₁₁ viral particles for every recombinant adenovirus in a dosage.	21 days between the rAd26 and rAd5 dosages	-18°C (liquid form) for up to 6 months at 2-8°C (freeze-dried).	100% 21 days following the initial dose	EUA:Russia, Belarus, Argentina, Serbia, UAE, Algeria, Palestine and Egypt
CoronaVac	Sinovac Biotech (China)	Inactivated virus vaccine	SARS-CoV-2 Inactivated CNO2 Strain produced using Vero cells	3 μg with an adjuvant of aluminum hydroxide	2 doses were separated 14 days apart.	2-8C; undetermined lifespan	Unknown	EUA: China, Brazil, Columbia, Uruguay, Turkey, Indonesia and Azerbaijan
BBIBP- CorV	Sinopharm 1/2 (China)	Inactivated virus Vaccine	SARS-CoV-2 Inactivated CNO2 Strain produced using Vero cells	4μg with an adjuvant of aluminum hydroxide	14-day intervals between the two dosages	2-8C; uncertain lifespan	Unknown	EUA: China, UAE, Bahrain, Serbia, Peru and Zimbabwe

Table 1. Different SARS-CoV-2 vaccines¹³

The primary issue with mRNA vaccines is formulation safety, as mRNA vaccines must be delivered and maintained at specific temperatures to avoid mRNA breakdown. Even if mRNA shots are a successful strategy to halt the COVID-19 pandemic, more study is needed to demonstrate the vaccine's persistence and protection.

3.2 Protein Subunit Vaccines

Subunit vaccines are better than whole-virion injections because there is a lower chance of contact during production. The corona virus is able to penetrate host cells thanks to the spike (S) proteins that are present on their surface and are commonly used as therapeutic and preventive targets¹⁶.

The NVX-CoV2373 vaccine fights the virus by using the full SARS-COV-2 spike glycoprotein, which is synthesized via the baculo virus-Spodoptera frugiperda (Sf9) insect cell translation process. According to study, using Matrix-M can increase immune responses by improving antigen presentation and uptake by antigenpresenting cells, as well as mobilizing, activating, and maturing critical immune cells.

In the initial stages of examining the immunogenicity and safety of NVX-CoV2373, baboons and cynomolgus macaques were employed as nonhuman primate models. The CD4 T cell response induced by this immunization mainly produces IFN-c, IL-2, and TNF-a. NVX-CoV2373 is anticipated to have an 89.7 % success rate, according to preliminary UK data. The UK strain (B.1.1.7) is resistant to NVX-CoV2373, according to a noteworthy finding from the same investigation¹⁷.

3.3 Adenovirus Vector-Based Vaccines

The current SARS-Coronavirus-2 vaccines use adenovirus DNA vectors. The genetic material in the vectors encodes for the SARS-COV-2 spike glycoprotein. Following the inoculation approach, the mRNA will soon start the inoculated population's production of the viral spike protein.

3.3.1. AZD1222

AZD1222 was developed in conjunction with AstraZeneca and the University of Oxford. The method uses an adenovirus that targets chimpanzees and has the genetic code for the SARS-CoV-2 spike protein. This study's viral vector was based on the low-replicationcapacity chimpanzee adenovirus¹⁰. Because of their high immunogenicity and stable genomes, chimp vectors are favoured for human vaccine research¹⁸. Despite getting only one dose of the immunization, they were still able to produce strong CD8-T cell and antibody responses. Chimpanzee adenovirus carriers pose no risk because they do not disrupt any pre-existing antibodies to human adenoviruses. The vaccine was effective against laboratoryconfirmed COVID-19 (71.42 %). The trial began 15 days following the second dose and continued for two months until the data collection requirement was reached. The vaccine was still effective (63.09 %) at this point¹⁹.

There have been reports of thrombocytopenia, hemor rhage, and arterial and venous thromboses related to the administration of the AZD1222 vaccine, with such events occurring between a few days to a few weeks post-immunization. In general, the AZD1222 Vaccine's safety, immunogenicity, and effectiveness results show promising potential.

3.3.2. Gam-COVID-Vac (Sputnik V)

The Gam-COVID-Vac (Sputnik V) vaccine was developed by the Gamaleya National Centre of Epidemiology and Microbiology, Moscow, using a heterologous recombinant adenovirus approach. For the initial and subsequent injections, the immunization technique uses two distinct adenovirus vector categories, called rAd26 and rAd5, respectively. The questioned vectors include the entire SARS-CoV-2 glycoprotein S gene²⁰.

The provided vaccination did not cause any notable adverse responses, according to the initial findings of phase 1 clinical studies. The frozen formulation outperformed the lyophilized formulation in terms of neutralizing antibody induction (49.25 vs. 45.95), IgG titre (14.703 vs. 11.143), CD4 (2.5 vs. 1.3) and CD8 (1.3 vs. 1.1) T cell proliferation rates, and neutralizing antibody levels.

The phase 3 trial participants were given two vaccination doses, one of rAD26-S and the other of rAD5-S. According to the phase's findings, the vaccine will most likely have a 91.6% efficacy rate. The spike protein produced by the B.1.1.7 and B.1.351 strains was successfully neutralized by serum from a volunteer who had received the Gam-COVID-Vac vaccination, according to a recent study ²¹.

3.4 Whole Virus Vaccines

One of the earliest techniques used to create vaccinations that prevented or treated viral infections in the past involved the inactivation of entire viruses²². Inactivated viral vaccinations are reportedly less dangerous than live attenuated virus vaccines, which contain the entire pathogen and may transform back into its original form and infect those with weakened immune systems²³.

3.4.1 Corona Vaccine

A SARS-CoV-2 immunological adjuvant, Corona Vac. (formerly PicoVacc), is also known as COVID-19 Vaccine (Vero Cell) Inactivated. The SARS-CoV-2 vaccination, specifically the CN2 strain, is also given. The vaccine regimen was developed by Sinovac Biotech Ltd. and consists of two doses of vaccine. The pathogenic bacterium was isolated from affected people's BALF and then multiplied in large Vero cell cultures. After being exposed to b-propiolactone for 24 hours, it became inactive. Ion-exchange chromatography and Size Exclusion Chromatography were then used to purify it. Finally, it was adsorbed onto an adjuvant of aluminum hydroxide²⁴.

Several Corona Vac. dosages and administration procedures were investigated based on the findings and found to have comparable levels of safety and immunogenicity²⁵. The study found that mild and moderate cases were completely successful. Furthermore, the efficiency against the need for assistance, as shown by a WHO Clinical Progression Scale value of 3. Corona Vac. had a 50 % efficacy rate in avoiding symptomatic COVID-19 after a minimum of 14 days following the second dose delivery, according to Palacios et al.'s investigation.

According to a study conducted in Turkey, 83.5 % (95 % CI 65.4-92.1) of laboratory-confirmed symptomatic COVID-19 people were resistant to Corona Vaccine. In experiments conducted in Indonesia and Brazil, Corona Vac. demonstrated a 65.3% (range: 20.0-85.1) effectiveness against symptomatic COVID-19²⁶.

3.4.2 Covilo; BBIBP-CorV

Sinopharm has created a pair of inactivated viral vaccines, the Sinopharm BIBP, which is specifically designed to tackle the COVID-19 virus. This study's findings are notable because they show that administering two 2 mg doses of the BBIBP-CorV vaccine can effectively prevent SARS-CoV-2 infection while not significantly increasing the rate of antibody-dependent transmission²⁷. A study comparing the efficacy of the AZD1222 vaccination and another vaccine discovered that both provided adequate protection against viral interstitial pneumonia in inoculated macaques. According to one study, the likelihood of adverse responses was low, and the vaccine displayed remarkable immunogenicity. According to early data from Bahrain and the UAE, BBIBP-CorV has a 100 % efficacy rate in the treatment of severe cases and a 78.1 % efficacy rate in the treatment of problematic patients²⁸.

3.4.3 BBV152 (COVAXIN)

According to one study, Bharat Biotech's Covaxin (BBV152) vaccine is 69 % effective. The vaccination in issue has been rendered inactive through the use of -propiolactone²⁹. Gallamide, a toll-type receptor 7/8 agonist imid-azo-quinoline connected to alum, is utilized as an adjuvant to target the disabled whole-virion structure (Algel-IMDG). The formulation has been proven to improve vaccination antigen localization to drainage lymph nodes while lowering systemic leakage. The Asp614Gly mutation is essential for the production of Covaxin and defines the features of this strain³⁰.

According to the findings, BBV152 caused increased immunological responses and a preference for T-helper-1 cells in T-cell reactions. According to physicians, the most prevalent side effects in the Phase II experiment were discomfort at the injection site, headaches, weariness, and increased body temperature. After two doses, phase III data for those with no prior history of infection show an intermediate efficiency of 81 % for avoiding COVID-19³¹. Emergency use authorization (EUA) and storage conditions for various vaccines are mentioned in table-1.

4. ANTIVIRAL DRUGS FOR SAR-COV-2

Corona viruses enter host cells by fusing and adhering to the membrane. The virus penetrates the host cell and stops replication with its own RNA-dependent RNA polymerase³². Sofosbuvir, a highly effective hepatitis C medication, was created by lowering RdRp with synthetic nucleoside and nucleotide analogs³³. Remdesivir has been proven to be effective. The viral RdRp4 active homolog, which enters cells and assembles, blocks viral replication by blocking the virus from reproducing. To restrict the influence of analogs, exoribonuclease, an enzyme present in coronaviruses, proofreads the RNA strand. Remdesivir has the ability to avoid editing³². While changed viruses are less infectious, remdesivir resistance may be conferred through laboratory-induced viral modification.

The FDA has approved molnupiravir, an antiviral drug, to treat SARS-CoV-2 infection. When a virus replicates, the antiviral medication inhibits the viral polymerase enzyme, allowing adenosine or guanosine to be incorporated into the viral genome. As a result, the virus's ability to spread is eventually lost. Several studies have proposed that the most recent omicron form of the FDA-approved antiviral medicine Paxlovid be used unrestrictedly to combat various types of infection³⁴.

5. CHALLENGES

Considering tight isolation and quarantine regulations in hospital settings, studies suggest that contact-based transmission of SARS-CoV-2 is common³⁵. A recent study indicated that a considerable proportion of patients, specifically 41 %, and medical professionals, specifically 29 %, had developed an infection³⁶. The hospital setting necessitates stringent surveillance owing to the grave concern posed by this mode of transmission.

SARS-CoV-2 re-infection requires the presentation of molecular proof of the virus at two independent time intervals, as well as viral genomic sequencing data. The existence of negative tests after a previous positive test usually indicates the likelihood of reinfection rather than permanent virus carriage^{25,37}. Due to the lack of routine sequencing capabilities in public health laboratories and hospitals, suspected re-infection cases must be prioritised based on clinical and laboratory standards before additional investigations can be conducted. To detect possible SARS-CoV-2 re infection cases, public health laboratories can apply a guideline technique developed by the Centres for Disease Control and Prevention (CDC)³⁸. The suggestions offered in this context recognize the possibility of RT-PCR test results remaining positive for an extended period after clinical symptoms have resolved, so addressing a substantial barrier in the diagnosis of re-infections³⁷.

6. FUTURE STRATEGIES

The impact of the COVID-19 pandemic on the global economy, healthcare, and medical infrastructure have prompted scientists to concentrate their efforts on developing and standardizing SARS-CoV-2 quick diagnostic tests and therapies³⁹. COVID-19 spreads by human-to-human contact,

necessitating the use of point-of-care testing procedures that do not necessitate the use of specialized facilities or expensive laboratory equipment⁴⁰. The development of nasal mucosa vaccinations, which boost mucosal immune responses and provide protection against infections by stopping viruses from growing and evading the immune system and vaccines, is a possible future strategy⁴¹. To get over this limitation, one approach is to produce mucosal vaccines in nano particles while taking into account the complex architecture of the human respiratory epithelial mucosa. Mucosal vaccines can be administered using niosomes or liposomes to improve penetration and retention time⁴².

The therapeutic potential of stem cell treatment against COVID-19 is being investigated in a number of studies and clinical trials. Notably, Cao and colleagues' mouse study revealed that the SARS-CoV-2 virus's entrance was restricted in MSCs. The researchers use three different types of MSCs to determine whether host epithelial cells have the angiotensin-converting enzyme (ACE2) and transmembrane serine protease 2 (TMPRSS2) receptors required for viral endocytosis: umbilical cord (UC-MSCs), placenta (PD-MSCs), and adipose-derived (AD-MSCs)⁴³. Although MSCs are unable to influence the expression of both receptors in mouse lung tissues, they are unable to stimulate the expression of both indicators (ACE2, TMPRSS2) in macrophages and epithelial cells under diverse inflammatory stressors. The study shows that the fictional SARS-CoV-2 virus was unable to infect MSCs and increased the risk of infection after therapy⁴⁴.

7. CONCLUSION

SAR-CoV-2 detection using nucleic acid. Functional nucleic acids, which include oligonucleotides and Nucleic Acid Enzymes (NAEs), have piqued the interest of the medical community due to their outstanding capacity to identify and catalyze the target day with an 89 % success rate. RT-PCR is a rapid method for detecting viral material. This method uses the virion, nucleocapsid, plaque, and ORF1ab regions to identify viral RNA. The application of LAMP may improve the mobility of rapid testing methodologies. After extracting RNA from the throat fluid and stones of SARS patients, cDNA was generated by reverse transcription, fragmented, and displayed using restriction imaging (RD) technology. Before hybridization, PCR-fragmented DNA was tagged with a Cy5 universal primer. Due to the increased time and effort required for this technology, faster PCR fusion tests that are comparable in reliability and frequently resemble genome sequencing have emerged. Innovative sensors and biosensors are being researched to improve the sensitivity of antigen-based immunoassays.

REFERENCES

 Fernandes, Q.; Inchakalody, V.P.; Merhi, M.; Mestiri, S.; Taib, N.; Moustafa Abo El-Ella, D.; Bedhiafi, T.; Raza, A.; Al-Zaidan, L.; O. Mohsen, M.; Ali Yousuf Al-Nesf, M.; Ait Hssain, A.; Mohamad Yassine & Dermime, S. Emerging COVID-19 variants and their impact on SARS-CoV-2 diagnosis, therapeutics and vaccines. *Annals of Medicine*, 2022, **54**, 524–40. doi: 10.1080/07853890.2022.2031274

- Chan, J.F.W.; Kok, K.H.; Zhu, Z.; Chu, H.; To, K.K.W.; Yuan, S. & Yuen K.Y. Genomic characterization of the 2019 novel human-pathogenic corona virus isolated from a patient with atypical pneumonia after visiting Wuhan. *Emerging Microbes and Infections*, + 2020, 9, 221–36. doi: 10.1080/22221751.2020.1719902
- Anderson, R.M.; Fraser, C.; Ghani, A.C.; Donnelly, C.A.; Riley, S.; Ferguson, N.M.; Leung, G.M.; Lam, T.H. & Hedley, A.J. Epidemiology, transmission dynamics and control of SARS: The 2002-2003 epidemic. *Philosophical transactions of the royal* society B:Biological Sciences, 2004, 359, 1091–105. doi: 10.1098/rstb.2004.1490
- Subbarao, K. The success of SARS-CoV-2 vaccines and challenges ahead. *Cell Host and Microbel*, 2021, 29, 1111–23. doi: 10.1016/j.chom.2021.06.016
- Tsang, N.N.Y.; So, H.C.; Ng, K.Y.; Cowling, B.J.; Leung, G.M. & Ip, D.K.M. Diagnostic performance of different sampling approaches for SARS-CoV-2 RT-PCR testing: A systematic review and metaanalysis. *The Lancet Infectious Diseases*, 2021, 21, 1233-45.

doi: 10.1016/S1473-3099(21)00146-8

 Tahan, S.; Parikh, B.A.; Wallace, M.A.; Burnham, C-A.D & Wang, D. SARS-CoV-2 E Gene variant alters analytical sensitivity. *J. of Clinical Microbiology*, 202, 59.

doi: 10.1128/jcm.00075-21

- Shi, H.; Ma, W.; Wu, Q.; Zhang, B.; Song, Y.; Guo, Q.; Weiwei, X.; Yan, W. & Wenling, Z. Design and application of 60 mer oligonucleotide microarray in SARS coronavirus detection. *Chinese Science Bulletin*, 2003, 48, 1165–9. doi: 10.1360/03wc0216
- Alpdagtas, S.; Ilhan, E.; Uysal, E.; Sengor, M.; Ustundag, C.B. & Gunduz, O. Evaluation of current diagnostic methods for COVID-19. *APL Bioengineering*, 2020, 4.

doi:10.1063/5.0021554

- Di Domenico, M.; De Rosa, A. & Boccellino, M. Detection of sars-cov-2 proteins using an elisa test. *Diagnostics*, 2021, **11**, 1–7. doi: 10.3390/diagnostics11040698.
- Falzone, L.; Gattuso, G.; Tsatsakis, A.; Spandidos, D.A. & Libra, M. Current and innovative methods for the diagnosis of COVID-19 infection (Review). *International J. of Molecular Medicine*, 2021, 47, 1-23.

doi: 10.3892/ijmm.2021.4933

 Eftekhari, A.; Alipour, M.; Chodari, L.; Dizaj, S.M.; Ardalan, M.R.; Samiei, M.; Sharifi, S.; Vahed, SZ.; Huseynova, I.; Khalilov, R.; Ahmadian, E. & Cucchiarini, M. A comprehensive review of detection methods for SARS-CoV-2. *Microorganisms*, 2021, **9**, 1–18.

doi: 10.3390/microorganisms9020232

 Moitra, P.; Alafeef, M.; Dighe, K.; Frieman, M.B. & Pan, D. Selective naked-eye detection of SARS-CoV-2 mediated by N Gene targeted antisense oligonucleotide capped plasmonic nanoparticles ACS Nano, 2020, 1, 7617–27.

doi: 10.1021/acsnano.0c03822

Creech, C.B.; Walker, S.C. & Samuels, R.J. SARS-CoV-2 Vaccines 2021. American Medical Association, 325, 2021–3.

doi: 10.1001/jama.2021.3199

- Polack, F.P.; Thomas, S.J.; Kitchin, N.; Absalon, J.; Gurtman, A.; Lockhart, S.; Perez, J.L.; Marci, G.P.; Moreira, E.D.; Zerbini, C.; Bailey, R.; Swanson, K.A.; Roychoudhury, S.; Koury, K.; Li, P.; Kalina, W.V.; Cooper, D.; Frenck, R.W.; Hammitt, L.L.; Türeci, O.; Nell, H.; Schaefer, A.; Ünal, S.; Tresnan, D.B.; D.V.M.; Mather, S.; Dormitzer, P.R.; Sahin, U.; Jansen, K.U. & Gruber, W.C. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. New England J. of Medicine, 2020, 383, 2603–15. doi: 10.1056/nejmoa2034577
- 15. Thompson, M.G.; Burgess, J.L.; Naleway, A.L.; Tyner, H.L.; Yoon, S.K.; Meece, J.; Olsho, L.E.W.; Caban-Martinez, A.J.; Fowlkes, A.; Lutrick, K.; Kuntz, J.L.; Dunnigan, K.; Odean, M. J.; Hegmann, K.T.; Stefanski, E.; Edwards, L.J.; Schaefer-Solle, N.; Grant, L.; Ellingson, K.; Groom, H.C.; Thiese, M. S.; Lynn; Wesley, M.G.; Lamberte, J. M.; Sun, X.; Michael, E.; Phillips, A. L.; Groover, K. D.; Yoo, Y.M.; Gerald, J.; Brown, R. T.; Herring, M.K.; Joseph, G.; Beitel, S.; Morrill, T.C.; Mak, J.; Rivers, P.; Harris, K.M.; Hunt, D.R.; Arvay, M.L.; Kutty, P.; Fry, A.M. & Gaglani, M. Interim Estimates of vaccine effectiveness of BNT162b2 and mRNA-1273 COVID-19 vaccines in preventing SARS-CoV-2 infection among health care personnel, first responders, and other essential and frontline workers-Eight U.S. locations, December 2020-March. MMWR Surveillance Summaries, 2021, 70, 495-500. doi: 10.15585/mmwr.mm7013e3
- Wang, Y.; Wang, L.; Cao, H. & Liu, C. SARS-CoV-2 S1 is superior to the RBD as a COVID-19 subunit vaccine antigen. J. of Medical Virology, 2021, 93, 892-8.

doi: 10.1002/jmv.26320

 Heath, P.T.; Galiza, E.P.; Baxter, D.N.; Boffito, M.; Browne, D.; Burns, F.; Chadwick D.R.; Clark, R.; Cosgrove, C.; Galloway, J.; Goodman, A.L.; Heer A.; Higham, A.; Iyengar S.; Jamal, A.; Jeanes, C.; Kalra, P.A.; Kyriakidou C.; McAuley D.F.; Meyrick, A.; Minassian, A.M.; Minton J.; Moore P.; Munsoor, I.; NichollsH.; Osanlou, O.; Packham, J.; Pretswell C.H.; San Francisco Ramos, A.; Saralaya, D.; Sheridan, R.P.; Smith, R.; Soiza, R.L.; Swift, P.A.; Thomson, E.C.; Turner, J.; Viljoen, M.E.; Albert, G.; Cho, I.; Dubovsky, F.; Glenn, G.; Rivers, J.; Robertson, A.; Smith, K.; & Toback, S. Safety and efficacy of NVX-CoV2373 covid-19 vaccine. *New England J. of Medicine*, 2021, **385**, 1172–83. doi: 10.1056/nejmoa2107659

- Folegatti, P.M.; Bellamy, D.; Roberts, R.; Powlson, J.; Edwards, N.J.; Mair, C.F.; Bowyer, G.; Poulton, I.; Mitton, C.H.; Green, N.; Berrie, E.; Lawrie, A.M.; Hill, A.V.S.; Ewer, K.J.; Hermon-Taylor, J.; & Gilbert, S.C. Safety and immunogenicity of a novel recombinant simian adenovirus ChAdOx2 as a vectored vaccine, 2019, 7, 1–12. doi: 10.3390/vaccines7020040
- Ewer, K.J.; Lambe, T.; Rollier, C.S.; Spencer, A.J.; Hill, A.V.S.; & Dorrell, L. Viral vectors as vaccine platforms: From immunogenicity to impact. *Current Opinion in Immunology*, 2016, **41**, 47–54. doi: 10.1016/j.coi.2016.05.014
- Montaltia, M.; Solda, G.; Di Valerioa, Z.; Salussoliaa, A.; Lenzib, J.; Forcellinic, M.; Barvasd, E.; Guttmannd, S.; Messinab, R.; Poluzzie, E.; Raschie, E.; Riccardif, R.; Fantinib, MP.; Faucia, G.L.; Gorib, D. ROCCA observational study: Early results on safety of Sputnik V vaccine (Gam-COVID-Vac) in the Republic of San Marino using active surveillance. *Clinical Medicine*, 2021, **38**, 4–10.

doi: 10.1016/j.eclinm.2021.101027

- 21. Logunov, D.Y.; Dolzhikova, I.V.; Shcheblyakov, D.V.; Tukhvatulin, A.I.; Zubkova, O.V.; Dzharullaeva, A.S.; Kovyrshina, A.V.; Lubenets, N.L.; Grousova, D.M.; Erokhova Alina, S.; Botikov, A.G.; Izhaeva, F.M.; Popova, O.; Ozharovskaya, T.A.; Esmagambetov, I.B.; Favorskaya, I.A.; Zrelkin, D.I.; Voronina, D.V.; Shcherbinin, D.N.; Semikhin, A.S.; Simakova, Y.V.; Tokarskaya, E.A.; Egorova, D.A.; Shmarov, M.M.; Nikitenko, N.A.; Gushchin, V.A.; Smolyarchuk, E,A.; Zyryanov, S.K.; Borisevich, S.V.; Naroditsky, B.S.; Gintsburg, A.L. Safety and efficacy of an rAd26 and rAd5 vector-based heterologous primeboost COVID-19 vaccine: An interim analysis of a randomised controlled phase 3 trial in Russia. The Lancet, 2021, 397, 671-81. doi: 10.1016/S0140-6736(21)00234-8
- Chung, J.R.; Flannery, B.; Ambrose, C.S.; Bégué, R.E.; Caspard, H.; DeMarcus, L.; Fowlkes, A.L.; Kersellius, G.; Steffens, A. & Fry, A.M.; Live attenuated and inactivated influenza vaccine effectiveness. *Pediatrics*, 2019, 143.

doi: 10.1542/peds.2018-2094

- Revenko, H.O. Strength of anti-diphtheria and antitetanus immunity in HIV-Infected adults. *Bulletin of Problems Biology and Medicine*, 2020II, 4, 178. doi: 10.29254/2077-4214-2020-4-158-178-182
- Gao, Q.; Bao, L.; Mao, H.; Wang, L.; Xu, K.; Yang, M.; Li, Y.; Zhu, L.; Wang, N.; Lv, Z.; Gao, H.; Ge, X.; Kan, B.; Hu, Y.; Liu, J.; Cai, F.; Jiang, D.; Yin, Y.; Qin, C.; Li, J.; Gong, X.; Lou, X.; Shi, W.; Wu, D.; Zhang, H.; Zhu, L.; Deng, W.; Li, Y.; Lu,

J.; Li, C.; Wang, X.; Yin, W.; Zhang, Y. & Qin, C. Development of an inactivated vaccine candidate for SARS-CoV-2. *Science*, 2020, **369**, 77–81. doi: 10.1126/science.abc1932

- Babiker, A.; Marvil, C.E.; Waggoner, J.J.; Collins, M.H. & Piantadosia, A. The importance and challenges of identifying SARS-CoV-2 Re-infections. J. of Clinical Microbiology, 2021, 59. doi: 10.1128/JCM.02769-20
- 26. Tanriover, M.D.; Doğanay, H.L.; Akova, M.; Güner, H.R.; Azap, A.; Akhan, S.; Köse, S.; Erdinç, F.S.; Akalın, E.H.; Tabak, O.F.; Pullukçu, H.; Batum, O.; Yavuz, S.S.; Turhan, O.; Yıldırmak, M.T.; Köksal, I.; Taşova, Y.; Korten, V.; Yılmaz, G.; Çelen, M.K.; Altın, S.; Celik, I.; Bayındır, Y.; Karaoğlan, I.; Yılmaz, A.; Özkul, A.; Gur, H. & Unal, S. Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (Corona Vaccine): Interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey. *The Lancet*, 2021, **398**, 213–22.

doi: 10.1016/S0140-6736(21)01429-X

- 27. Qin, C; Zhou, L; Hu, Z; Zhang, S; Yang, S; Tao, Y; Xie, C; Ma, K; Shang, K; Wang, W. & Tian D.S. Dysregulation of immune response in patients with corona virus 2019 (COVID-19) in Wuhan, China. *Clinical Infectious Diseases*, 2020, **71**, 762–8. doi: 10.1093/cid/ciaa248
- Al Kaabi, N.; Zhang, Y.; Xia, S.; Yang, Y.; Qahtani, M.M.; Abdulrazzaq, N.; Nusair, M.A.; Hassany, M.; Jawad, J.S.; Abdalla, J.; Hussein, S.E.; Mazrouei, S.K. A.; Karam, M,A.; Li, X.; Yang, X.; Wang, W.; Lai, B.; Chen, W.; Huang, S.; QianWang; Yang, T.; Liu, Y.; Ma, R.; Hussain, Z.M.; Khan, T.; Fasihuddin, M.D.; You, W.; Xie, Z.; Zhao, Y.; Jiang, Z.; Zhao, G.; Zhang, Y.; Mahmoud, S.; ElTantawy, I.; Xiao, P.; Koshy, A.; Zaher, W.A.; Wang, H.; Duan, K. & Yang, X. Effect of 2 inactivated SARS-CoV-2 vaccines on symptomatic COVID-19 Infection in adults: A randomized clinical trial. JAMA - Journal of the American Medical Association, 2021, 326, 35–45. doi: 10.1001/jama.2021.8565
- Kumar, A.; Dowling, W.E.; Román, R.G.; Chaudhari, A.; Gurry, C.; Le T.T.; Tollefson, S.; Clark, C.E.; Bernasconi, V. & Kristiansen, P. Status report on COVID-19 vaccines development. *Current Infectious Disease Reports*, 2021, 23. doi: 10.1007/s11908-021-00752-3
- Shell, A. Evaluation of safety and immunogenicity of an adjuvanted, TH-1 skewed, whole virion inactivated SARS-CoV-2 vaccine - BBV152, 2016, 1–23. doi: 10.1101/2020.09.09.285445
- Darbar, S.; Agarwal, S.; Saha, S. COVID-19 Vaccine: COVAXIN ®-India's First Indigenous Effective Weapon to Fight against Corona virus (A Review). Parana Journal of Science and Education, 2021, 7, 1-9.

tiny.cc/PJSE24476153v7i3p001-009

- Amirian, E.S.; Levy, J.K. Current knowledge about the antiviral Remdesivir (GS-5734) and GS-441524 as therapeutic options for coronaviruses. *One Health*, 2020, 9, 100128. doi: 10.1016/j.onehlt.2020.100128
- 33. Ferner, R.E. & Aronson, J.K.; Remdesivir in covid-19. *The BMJ*, 2020, 369, 1–2. doi: 10.1136/bmj.m1610
- Kabinger, F.; Stiller, C.; Schmitzová, J.; Dienemann, C.; Kokic, G.; Hillen, H.S.; Hobartner, C.; Cramer, P. Mechanism of molnupiravir-induced SARS-CoV-2 mutagenesis. *Nature Structural and Molecular Biology*, 2021, 28, 740-6. doi: 10.1038/s41594-021-00651-0
- 35. Chan, J.F.W.; Yuan, S.; Kok, K.H.; Wang To, K.K.; Chu, H.; Yang, J.; Xing, F.; JLiu, J.; Yip, C.C.Y.; Poon, R.W.S.; Tsoi, H.W.; Lo, S.K.F.; Chan, K.H.; Poon, V.K.M.; Chan, W.M.; Daniel Ip, J.; Cai, J.P.; Cheng, V.C.; Chen, H.; Hui, C.K.M. & Yuen, K.Y. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: A study of a family cluster. *The* Lancet, 2020, **395**, 514–23.
- doi: 10.1016/S0140-6736(20)30154-9
 36. Wang, D.; Bo, Hu.; Chang, Hu.; Fangfang, Zhu.; Xing, Liu.; Jing, Zhang.; BinbinWan.; Hui Xiang.; Zhenshun Cheng.; Yong Xiong.; Yan, Zhao.; Yirong, Li.; XinghuanWang.; Zhiyong, Peng. Clinical characteristics of 138 hospitalized patients with 2019 novel corona virus-infected Pneumonia in Wuhan, China. JAMA -Journal of The American Medical Association, 2020, 323, 1061-9.
 - doi: 10.1001/jama.2020.1585
- Zhou, B.; She, J.; Wang, Y.; Ma, X. Duration of viral shedding of discharged patients with severe COVID-19. *Clinical Infectious Diseases*, 2020, **71**, 2240–2. doi: 10.1093/cid/ciaa451
- Carmol, A.; Pereira-Vaz1, J.; Mota, V.; Mendes, A.; Morais, C.; da Silva, A.C.; Camilo, E.; Pinto, C.S.; Cunha, E.; Pereira, J.; Coucelo, M.; Martinho, P.; Correia, L.; Marques, G.; Araújo, L.; Rodrigues, F. Clearance and persistence of SARS-CoV-2 RNA in patients with COVID-19. *Journal of Medical Virology*, 2020, 92, 2227–31.

doi: 10.1002/jmv.26103

- Varahachalam, S.P.; Lahooti, B.; Chamaneh, M.; Bagchi, S.; Chhibber, T.; Morris, K. Nanomedicine for the SARS-CoV-2: State-of-theart and future prospects. *International Journal of Nanomedicine*, 2021, 16, 539–60. doi: 10.2147/IJN.S283686
- Ajeet Kumar Kaushik.; Jaspreet Singh Dhau.; Hardik Gohel.; Yogendra Kumar Mishra.; Babak Kateb.; Nam-Young Kim & D.Y.G. Electrochemical SARS-CoV-2 sensing at point-of-care and artificial intelligence for intelligent COVID-19 management. ACS Applied Bio Materials, 2020, 3, 7306–25. doi: 10.1021/acsabm.0c01004

- Adesokan, A.; Obeid, M.A.; Lawal, A.F. SARS-CoV-2: Vaccinology and emerging therapeutics, challenges and future developments. *Therapeutic Delivery*, 2022, 13, 187–203. doi: 10.415/tde-2021-0075
- Evelina, Miele; Gian, Paolo, Spinelli; Ermanno, Miele; Enzo, Di, Fabrizio; Elisabetta, Ferretti, S.T. & A.G. Nano particle-based delivery of small interfering RNA: Challenges for cancer therapy. *International Journal of Nanomedicine*, 2012, 7, 3637–57. doi: 10.2147/IJN.S23696.
- Yajun Cao; Hongyan Wub; Wanli Zhai; Ying Wang; Mengdi Li; Meng Li; Liu Yang; Ye Tian; Yunhao Song; Jun Li; Yinyin Wang; Qiang Ding; Linqi Zhang; Ming Cai; Zhijie Chang. A safety consideration of mesenchymal stem cell therapy on COVID-19: Stem. Cell Research, 2020, 49, 102066. doi: 10.1016/j.scr.2020.102066
- 44. Vishal Khandelwal; Tarubala Sharma; Saurabh Gupta; Shoorvir Singh; Manish Kumar Sharma; Deepak Parashar; Vivek K. Kashyap. Stem cell therapy: A novel approach against emerging and re-emerging viral infections with special reference to SARS-CoV-2: *Molecular Biology Reports*, 2023, **50**, 2663–2683. doi: 10.1007/s11033-022-07957-2

CONTRIBUTORS

Ms Deepika Kumari is currently pursuing has MTech in Biotechnology from Noida International University, Greater Noida. She has contributed to data and manuscript preparation. **Dr Anil Kumar Mavi** received his PhD in Biomedical Sciences, Faculty of Medical Sciences Vallabhbhai Patel Chest University of, University of Delhi, Delhi. Currently, He is working as Assistant Professor in Department of Botany & Life Science, Sri Aurobindo College, University of Delhi.

He has contributed to data collection and manuscript preparation.

Ms Jyoti Chaudhary is currently working as Assistant Professor in Noida International University, Greater Noida. She holds PhD from department of Biotechnology, Amity University. She has contributed guidance and framing research study.

Dr Manoj Kumar is currently working as Senior Technical Assistant in Vallabhbai Patel Chest Institute, University of Delhi. His research interests lie in non communicable diseases and association of natural products to treat these diseases. He has conceived the study, designed the experiment and contributed to manuscript preparation.

Dr Rajesh Kumar Gupta received PhD in Chemistry from University of Delhi. Currently, He is working as an Assistant Professor in Gautam Buddha University.

His research interest lies medicinal chemistry, nutritional biochemistry, antioxidant activity, tuberculosis. He has contributed in the image preparation.

Dr Umesh Kumar obtained his PhD (Epigenetics in Breast Cancer) from Dr B.R. Ambedkar Center for Biomedical Research, University of Delhi. Currently, He is working as Head, Research & Associate Professor in School of Biosciences, IMS Ghaziabad University Courses Campus, Ghaziabad. His keen interest is in HPV infection, Breast cancer epigenetics, stem cell biology & Molecular medicine.

He has contributed towards manuscript preparation, conceived the study data collection and literature analysis.