White Paper

UV/Vis Spectrophotometry Applications
in COVID-19 Vaccine Research

An overview of in-process COVID-19 vaccine research and how UV/VIS Excellence spectrophotometers can increase speed and accuracy when characterizing vaccine candidates and their components.

Contents

METTLER TOLEDO

White Paper

1. Introduction

A vaccine is a type of treatment aimed at stimulating the body's immune system to fight against infectious pathogens such as bacteria and viruses. They are, according to the World Health Organization, "one of the most effective ways to prevent disease".

1.a. Vaccine components

The important ingredients of any vaccine designed to create an active immunization are:

- **Antigens:** weakened versions of live viruses, e.g. parts of the virus envelope or virus genetic information
- **Adjuvants:** components intended to amplify the immune response to an antigen
- **Preservatives:** substances that ensure the stability of vaccines, e.g. the compound thiomersal (thimerosal)

1.b. Vaccine manufacturing

The common process for manufacturing a vaccine involves three phases, as noted below.

1. **Bioprocessing.** A process that uses complete living cells such as bacteria, viruses, or enzymes to obtain a desired product. This process has three main processing stages:
   - Upstream processing. This includes culture isolation, cultivation and collection of the live cell batch.
   - Midstream processing. This process includes the removal of cells and cell debris that typically takes place between fermentation (upstream) and product purification (downstream).
   - Downstream processing. The process whereby a cell mass generated upstream is processed to meet purity and quality requirements.
2. **Formulation, filling and quality control**
3. **Finishing and packaging**

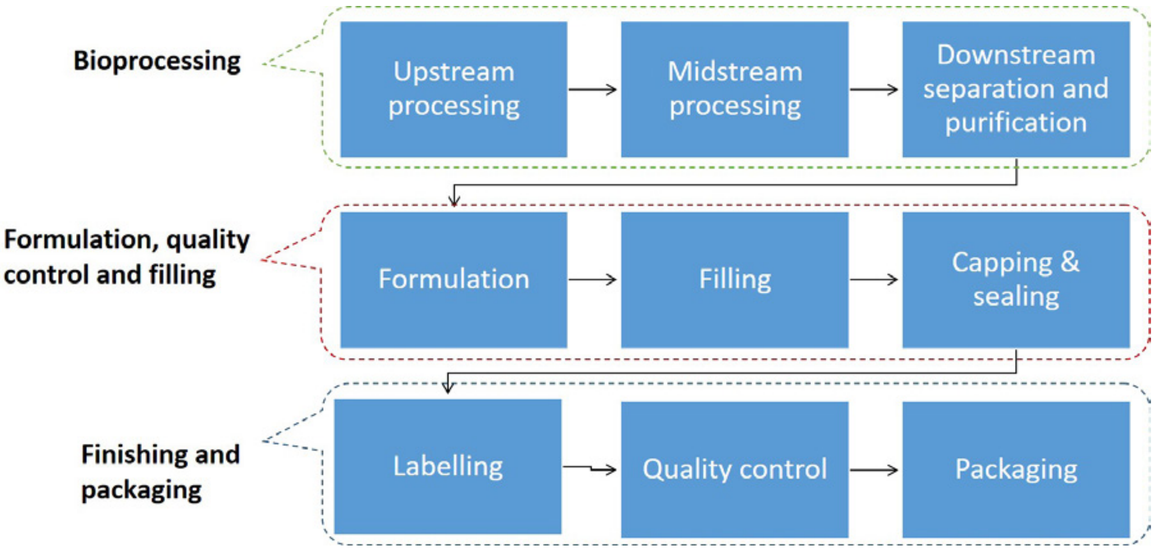

Fig 1. Overview of vaccine manufacturing process

As noted in the following chapters, UV/Vis spectrophotometry provides fast, easy and accurate characterization of components such as nucleic acids, proteins and additives/preservatives and can impact the time-to-result for both downstream and upstream processing, including quality control.

1.c. Urgency around COVID-19 vaccine development

The head lab technician in the Pathology Department of Sir J. J. Hospital, Mumbai, was contacted to gain an understanding of the current treatments for live cases of COVID-19. This hospital is Mumbai's best multi-specialty government-run public hospital and includes an RT-PCR laboratory that is the largest COVID-19 testing facility in India.

It was confirmed that there is no specific medication available that has a targeted effect on COVID-19. Patients are currently treated with a combination of antimalarial and anti-HIV drugs, namely chloroquine, hydroxychloroquine, lopinavir, and remdesivir. This lack of targeted treatment along with the infectious and potentially virulent nature of COVID-19 lends urgency to the search for an effective vaccine.

2. Current coronavirus vaccine research

Currently (May 2020), worldwide there are at least 117 studies in process to develop a vaccine for coronavirus. For a list of these projects, please see the Annex.
To develop a vaccine that targets SARS-CoV-2, scientists are working on a range of models. Researchers who have been able to map these models in 3D suggest that a viable antigen could be a promising way of stimulating an immune response. Types of coronavirus vaccine models currently being researched follow (Table 1).

Table 1. Types of active coronavirus vaccines under research

No.	Coronavirus Vaccine	Description
1	Inactivated vaccines	Dead or killed vaccine consisting of virus particles
2	Live attenuated vaccines	Weakened (or attenuated) form of the coronavirus genome that causes a disease
3	S protein-based vaccines	Vaccines containing recombinant S protein
4	Vectored vaccines	Chemically weakened virus that transports the pathogen
5	DNA-based vaccines	DNA that codes for specific protein antigens
6	Combination vaccines	Two or more individual vaccines given as one

Similar to SARS and MERS, the 2019-nCoV genome encodes non-structural proteins (such as 3-chymotrypsin-like protease, papain-like protease, helicase, and RNA-dependent RNA polymerase), structural proteins (such as spike glycoprotein) and accessory proteins, as shown in Figure 2. UV/Vis spectrophotometry can assist with the rapid and accurate analysis of many of these compounds, as noted in the chapters that follow.

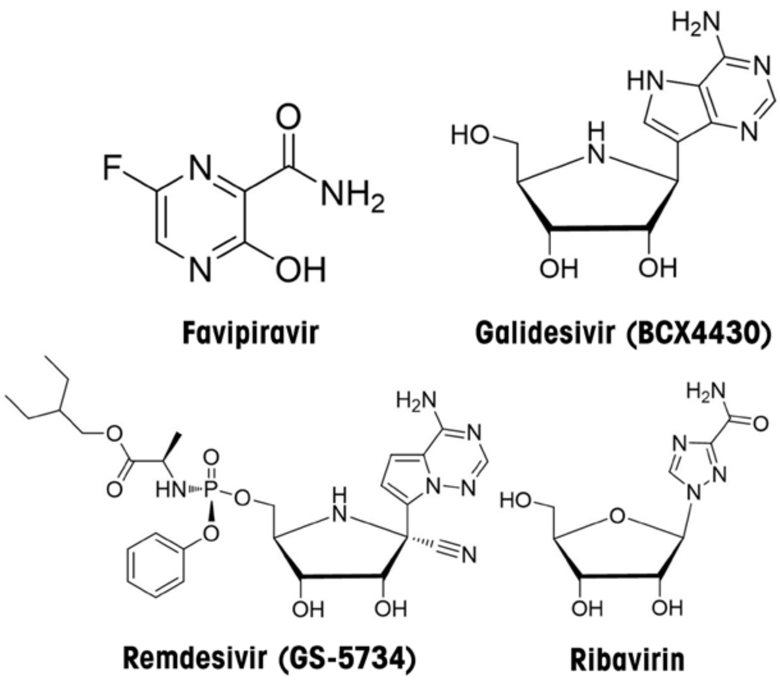

Figure 2: Chemical structures of four potential inhibitors interfering with the RNA polymerase of 2019-nCoV

3. UV/Vis spectrophotometry in COVID-19 vaccine analysis

Normally, vaccine development can take years. However, because COVID-19 can present with potentially life-threatening symptoms and currently has no targeted treatment, many labs worldwide are working to safely shorten the typical vaccine development timeline in an effort to prevent additional deaths.

As mentioned earlier (Table 1), the vaccines under development for COVID-19 will consist of the three components typically required to create an immune system response:

- nucleic acids, i.e. DNA/RNA
- protein
- additives (i.e. preservatives)

Some of a lab's ability to shorten time-to-result and therefore time-to-market depends on the type of analysis criteria being considered and timely, accurate characterization of the type of antigen being studied, whether protein, virus, bacterium, or nucleic acid.

The METTLER TOLEDO UV/VIS Excellence spectrophotometers UV5Nano and UV5Bio can be important tools for obtaining accurate, reliable and fast quantification. They can also provide purity checks of the above listed components as illustrated in Figure 3.

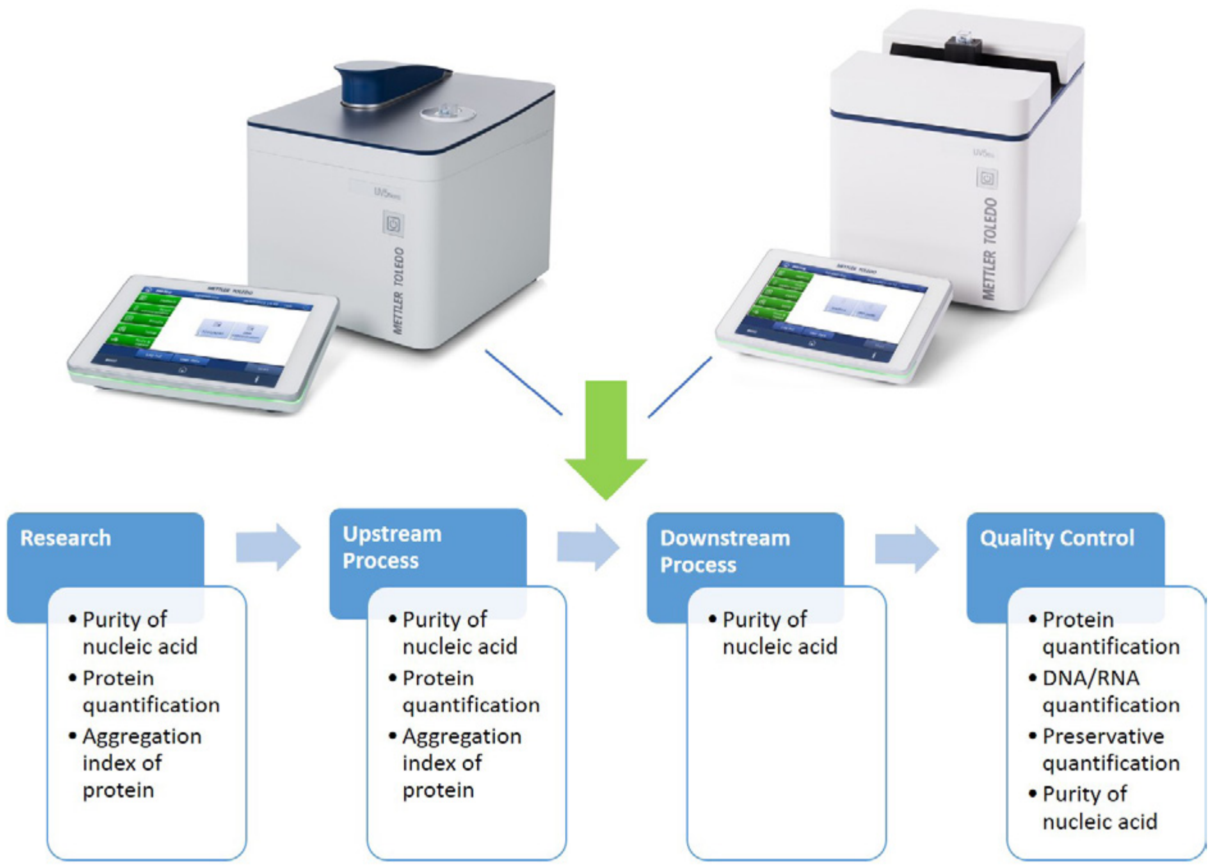

Fig. 3. UV/Vis applications for vaccine analyses

3.a. Ready-to-use applications in COVID-19 research

The METTLER TOLEDO ready-to-use applications listed below have proven to be of significant benefit to the vaccine development process during research, upstream processing, downstream processing, and quality control (Table 2). These applications are expected to play a role in the development of the COVID-19 vaccine as well.

Table 2. METTLER TOLEDO ready-to-use applications available in UV5Bio and UV5Nano for vaccine analyses

Parameter	Method ID	Test
Protein quantification	M9107	Biuret
	M9105	Lowry
	M9101	Bradford
	M9106	BCA
Purity and concentration of DNA/RNA	M9501	Purity and concentration of nucleic acids

3.b. General UV/Vis spectroscopy applications

UV/Vis spectroscopy is currently used to analyze a variety of critical vaccines including influenza and rabies. This technology is also applicable to the search for the COVID-19 vaccine.

Common parameters currently analyzed using UV/Vis spectroscopy for existing vaccines are listed in Table 3.

Table 3. UV/Vis used in vaccine research and analysis (non-COVID-19)

No.	Vaccine component	Compound/Parameter analyzed by UV/Vis	Ref.
1	Detoxification agent in vaccines	Formalin	[2]
2	Vaccine preservative, Hib-TT conjugate vaccine	Thiomersal (thimerosal)	[2]
3	Rabies vaccine	Protein content by biuret	[2]
4	Vaccine preservative	Phenol	[2]
5	Split virus influenza vaccine	Triton-100	[3]
6	Typhoid fever vaccine	Capsular polysaccharide of Salmonella typhi Vi and Vi protein conjugate	[4]
7	Nucleic acid in polysaccharides vaccines	High-molecular-weight polysaccharide antigens, nucleic acids	[5]
8	Herpes simplex virus 2 DNA vaccine	Total nucleic acid concentration and plasmid DNA purity	[6]
9	Purification of avian influenza virus	A260/A280	[7]
10	SARS-CoV vaccine	Protein BCA assay	[8]
11	Glyco-conjugate vaccines containing exopolysaccharide	Hydrolytic activity i.e. enzyme kinetic assay	[9]
12	Anticancer therapeutics	Conjugation and interaction between bleomycin and Au nanoparticles	[10]
13	MERS-CoV vaccine	Colorimetric assay of Au nanoparticles	[11]

4. Selected UV/Vis applications

What follows are application details for three selected vaccine components used as preservatives (Table 3). The application examples show how UV/Vis spectrophotometry can be used for accurate quantification.

- **Thiomersal (thimerosal).** The common method of quantifying this vaccine preservative is its extraction in organic solvent (chloroform) followed measuring the absorbance of the organic layer at 490 nm.

- **Formalin.** The content of this detoxifying agent can be determined by colorimetric analysis based on the reaction of formalin with methylbenzothiazolone hydrazone hydrochloride. The method yields a color complex whose absorbance can be measured at 628 nm.

- **Phenol.** This vaccine preservative can be reacted with 4-aminoantipyrine to provide a color complex. The absorbance of this complex can be measured at 550 nm to correlate with the concentration of phenol present.

5. Conclusion

- Currently (May 2020), there are at least 117 COVID-19 vaccines in development around the world. Most of these are DNA or RNA vaccines. However, as of now, no vaccine made from genetic material such as DNA or RNA has been approved to date.
- Among all the listed vaccines, only eight are under clinical evaluation while the remaining vaccine candidates are still in pre-clinical trials.
- In addition to the limited number of COVID-19 vaccine candidates in the pipeline, no drugs are available for prevention, prophylaxis and treatment of coronavirus infections in humans.
- METTLER TOLEDO UV/Vis can be a useful tool in vaccine research during development and synthesis, and it can also be used in the analysis of viruses themselves. This technology helps to quickly and accurately characterize major vaccine constituents, including DNA, RNA, specific proteins, and RNA inhibitors.

6. List of abbreviations

CoV	Coronavirus
SARS	Severe Acute Respiratory Syndrome
MERS	Middle East Respiratory Syndrome
BCA	Bicinchoninic acid assay

7. References

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8. Annex

1. Vaccines under clinical and pre-clinical evaluation (primarily involving biopharmaceutical, biotechnology, or research organizations) [1]

- Vaccines Under Clinical Evaluation

No.	Platform	Candidate type	Developerw	Applicability of UV/Vis Spectroscopy
1	Non-Replicating Viral Vector	Adenovirus type 5 vector	CanSino Biological Inc. and Beijing Institute of Biotechnology	
2	RNA	LNP-encapsulated mRNA	Moderna/NIAID	✓
3	DNA	DNA plasmid vaccine with electroporation	Inovio Pharmaceuticals	✓
4	Inactivated	Inactivated	Beijing Institute of Biological Products/ Sinopharm	
5	Inactivated	Inactivated + alum	Sinovac	
6	Non-Replicating Viral Vector	ChAdOx1	University of Oxford	
7	RNA	3 LNP-mRNAs	BioNTech/Fosun Pharma/Pfizer	✓
8	Inactivated	Inactivated	Wuhan Institute of Biological Products/Sinopharm	

- Vaccines in Pre-Clinical Evaluation

No.	Platform	Candidate type	Developer	Applicability of UV/Vis Spectros-copy
1	DNA	DNA	Takis/Applied DNA Sciences/Evvivax	✓
2	DNA	DNA plasmid vaccine	Zydus Cadila	✓
3	DNA	DNA with electroporation	Karolinska Institute/ Cobra Biologics (OPENCO-RONA Project)	✓
4	DNA	DNA plasmid vaccine	Osaka University/AnGes/ Takara Bio	✓
5	DNA	Plasmid DNA, needle-free delivery	Immunomic Therapeutics, Inc./EpiVax, Inc./ PharmaJet, Inc.	✓
6	DNA	DNA plasmid vaccine	BioNet Asia	✓
7	DNA	DNA vaccine	University of Waterloo	✓
8	DNA	DNA vaccine	Entos Pharmaceuticals	✓
9	DNA	bacTRL-Spike	Symvivo	✓
10	Inactivated	TBD	Osaka University/BIKEN/ NIBIOHN	
11	Inactivated	Inactivated	Institute of Medical Biology , Chinese Academy of Medical Sciences	
12	Inactivated	Inactivated + CpG 1018	Sinovac/Dynavax	
13	Inactivated	Inactivated + CpG 1018	Valneva/Dynavax	
14	Inactivated	Inactivated	Research Institute for Biological Safety Prob-lems, Rep of Kazakhstan	
15	Live Attenu-ated Virus	Condon deoptimized live attenuated vac-cines	Codagenix/Serum Institute of India	
16	Live Attenu-ated Virus	Condon deoptimized live attenuated vac-cines	Indian Immunologicals Ltd/Griffith University	
17	Live Attenu-ated Virus	Measles Virus (S, N targets)	DZIF – German Center for Infection Research	
18	Non-Repli-cating Viral Vector	MVA encoded VLP	GeoVax/BravoVax	
19	Non-Repli-cating Viral Vector	Ad26 (alone or with MVA boost)	Janssen Pharmaceutical Companies	
20	Non-Repli-cating Viral Vector	Adenovirus-based NasoVAX expressing SARS2-CoV spike protein	Altimmune	
21	Non-Repli-cating Viral Vector	Oral Vaccine platform	Vaxart	
22	Non-Repli-cating Viral Vector	Replication defective simian adenovirus (GRAd) encoding SARS-CoV-2 S	ReiThera/LEUKOCARE/Univercells	
23	Non-Repli-cating Viral Vector	MVA-S encoded	DZIF – German Center for Infection Research	
24	Non-Repli-cating Viral Vector	Ad5 S (GREVAX™ platform)	Greffex	
25	Non-Repli-cating Viral Vector	MVA-expressing structural proteins	Centro Nacional Biotecnología (CNB-CSIC), Spain	
26	Non-Repli-cating Viral Vector	Dendritic cell-based vaccine	University of Manitoba	
27	Non-Repli-cating Viral Vector	Oral Ad5 S	Stabilitech Biopharma Ltd	
28	Non-Repli-cating Viral Vector	adenovirus-based + HLA-matched pep-tides	Valo Therapeutics Ltd	
29	Non-Repli-cating Viral Vector	parainfluenza virus 5 (PIV5)-based vac-cine expressing the spike protein	University of Georgia/University of Iowa	
30	Non-Repli-cating Viral Vector	Recombinant deactivated rabies virus containing S1	Bharat Biotech/Thomas Jefferson University	

No.	Platform	Candidate type	Developer	Applicability of UV/Vis Spectros-copy
31	Protein Sub-unit	Capsid-like particle	AdaptVac (PREVENT-nCoV consortium)	✓
32		Drosophila S2 insect cell expression sys-tem VLPs	ExpreS2ion	✓
33		Peptide antigens formulated in lipid nanoparticle formulation	IMV Inc.	✓
34		S protein	WRAIR/USAMRIID	✓
35		Native like Trimeric subunit Spike Protein vaccine	Clover Biopharmaceuticals Inc./GSK/Dynavax	✓
36		S protein + adjuvant	National Institute of Infectious Disease, Japan	✓
37		VLP-recombinant protein + adjuvant	Osaka University/ BIKEN/ National Institutes of Biomedical Innovation, Japan	✓
38		Microneedle arrays S1 subunit	University of Pittsburgh	✓
39		Peptide	Vaxil Bio	✓
40		Adjuvanted protein subunit (RBD)	Biological E. Limited	✓
41		Peptide	Flow Pharma, Inc.	✓
42		S protein	AJ Vaccines	✓
43		Ii-key peptide	Generex/EpiVax	
44		S protein	EpiVax/University of Georgia	✓
45		S protein (baculovirus production)	Sanofi Pasteur/GSK	✓
46		Full length recombinant SARs CoV-2 gly-coprotein nanoparticle vaccine adjuvanted with Matrix M	Novavax	✓
47		gp-96 backbone	Heat Biologics/University of Miami	✓
48		Molecular clamp-stabilized spike protein	University of Queensland/GSK/Dynavax	✓
49		S1 or RBD protein	Baylor College of Medicine	✓
50		Subunit protein, plant produced	iBio/CC-Pharming	✓
51		Recombinant protein, nanoparticles (based on S-protein and other epitopes)	St. Petersburg Scientific Research Institute of Vaccines and Serums	✓
52		COVID-19 XWG-03 truncated S (spike) proteins	Innovax/Xiamen Univ./GSK	✓
53		Synthetic Long Peptide Vaccine candidate for S and M proteins	OncoGen	✓
54		Oral E. coli-based protein expression sys-tem of S and N proteins	MIGAL Galilee Research Institute	✓
55		Spike-based	University of Alberta	✓
56		Adjuvanted microsphere peptide	VIDO-InterVac, University of Saskatchewan	✓
57		Peptide vaccine	FBRI SRC VB VECTOR, Rospotrebnadzor, Koltsovo	✓
58		Subunit vaccine	FBRI SRC VB VECTOR, Rospotrebnadzor, Koltsovo	✓
59		Nanoparticle vaccine	LakePharma, Inc.	✓
60		Recombinant spike protein with Advax™ adjuvant	Vaxine Pty Ltd	✓

No.	Platform	Candidate type	Developer	Applicability of UV/Vis Spectros-copy
61	Protein Sub-unit	OMV-based vaccine	Quadram Institute Biosciences	✓
62		OMV-based vaccine	BiOMViS Srl/Univ. of Trento	✓
63		Recombinant S1-Fc fusion protein	AnyGo Technology	✓
64		Recombinant protein	Yisheng Biopharma	✓
65		Recombinant S protein in IC-BEVS	Vabiotech	✓
66		Orally delivered, heat stable subunit	Applied Biotechnology Institute, Inc.	✓
67		S-2P protein + CpG 1018	Medigen Vaccine Biologics Corporation/NIAID/ Dynavax	✓
68		Peptides derived from Spike protein	Axon Neuroscience SE	✓
69		Adjuvanted recombinant protein (RBD-Dimer)	Anhui Zhifei Longcom Biopharmaceutical/ Institute of Microbiology, Chinese Academy of Sciences	✓
70		RBD-based	Neovii/Tel Aviv University	✓
71		Outer Membrane Vesicle (OMV)-peptide	Intravacc/Epivax	✓
72	Replicating Viral Vector	Measles vector	Zydus Cadila	
73		YF17D Vector	KU Leuven	
74		Measles vector	Institute Pasteur/Themis/ University of Pittsburg Center for Vaccine Research	
75		Horsepox vector expressing S protein	ITonix Pharma/Southern Research	
76		Live viral-vectored based on attenuated influenza virus backbone (intranasal)	BiOCAD and IEM	
77		Influenza vector-expressing RBD	University of Hong Kong	
78		Replication-competent VSV chimeric virus technology (VSVΔG) delivering the SARS-CoV-2 spike (S) glycoprotein	IAVI/Batavia	
79		VSV-S	University of Western Ontario	
80		Measles Vector	FBRI SRC VB VECTOR, Rospotrebnadzor, Koltsovo	
81		Recombinant vaccine based on Influenza A virus, for the prevention of COVID-19 (intranasal)	FBRI SRC VB VECTOR, Rospotrebnadzor, Koltsovo	
82		VSV vector	FBRI SRC VB VECTOR, Rospotrebnadzor, Koltsovo	
83		M2-deficient single replication (M2SR) influenza vector	UW–Madison/FluGen/Bharat Biotech	
84		Newcastle disease virus vector (NDV-SARS-CoV-2/Spike)	Intravacc/ Wageningen Bioveterinary Research/ Utrecht Univ.	
85		Avian paramyxovirus vector (APMV)	The Lancaster University, UK	

No.	Platform	Candidate type	Developer	Applicability of UV/Vis Spectros-copy
86	RNA	LNP-encapsulated mRNA cocktail encoding VLP	Fudan University/ Shanghai JiaoTong University/ RNACure Biopharma	✓
87		LNP-encapsulated mRNA encoding RBD	Fudan University/ Shanghai JiaoTong University/ RNACure Biopharma	✓
88		Replicating defective SARS-CoV-2 derived RNAs	Centro Nacional Biotecnología (CNB-CSIC), Spain	✓
89		LNP-encapsulated mRNA	University of Tokyo/ Daiichi Sankyo	✓
90		Liposome-encapsulated mRNA	BIOCAD	✓
91		mRNA	China CDC/Tongji University/ Stermina	✓
92		mRNA	Arcturus Therapeutics /Duke-NUS	✓
93		mRNA	Curevac	✓
94		saRNA	Imperial College London	✓
95		LNP-mRNA	Translate Bio/Sanofi Pasteur	✓
96		Several mRNA candidates	RNAimmune, Inc.	✓
97		mRNA	FBRI SRC VB VECTOR, Rospotrebnadzor, Koltsovo	✓
98		mRNA in an intranasal delivery system	eTheRNA	✓
99		mRNA	Greenlight Biosciences	✓
100	VLP	Plant-derived VLP	Medicago, Inc.	
101		Virus-like particle based on RBD displayed on virus-like particles	Saiba GmbH	
102		ADDomerTM multi-epitope display	Imophoron Ltd. and Bristol University's Max Planck Centre	
103		Unknown	Doherty Institute	
104		VLP	OSIVAX	
105		eVLP	ARTES Biotechnology	
106		VLPs peptides/whole virus	Univ. of Sao Paulo	
107	Unknown	Unknown	Tulane University	
108	Unknown	Unknown	ImmunoPrecise	
109	Unknown	Unknown	Université Laval	

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