BHARATHIDASAN UNIVERSITY

Tiruchirappalli- 620024,
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Programme: M.Sc., Biomedical science

Course Title : Molecular medicine

Course Code : BM48C16M

Unit-V

TOPIC: SARS-CoV-2

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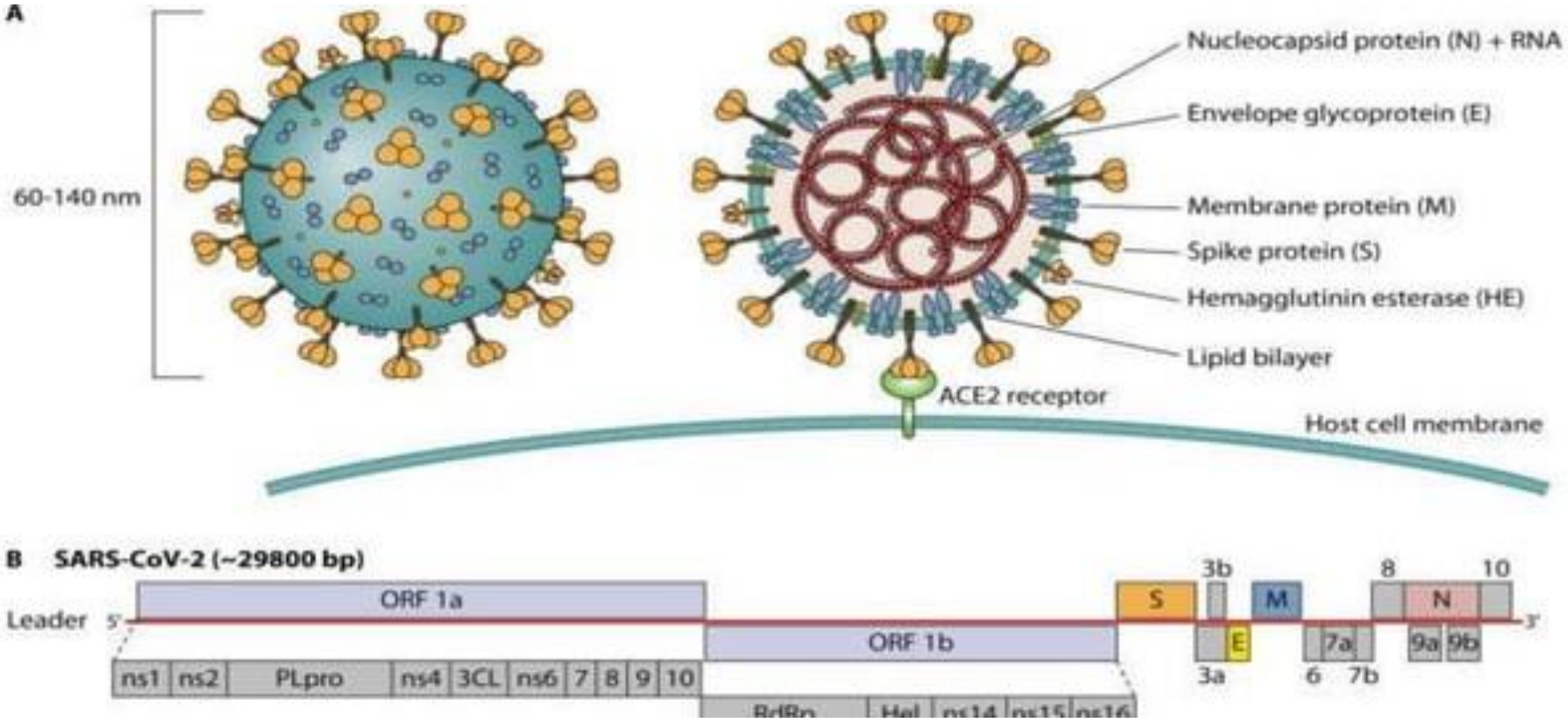
SARS-COV-2


History of COVID-19
December 2019
1st Case of COVID-19
January 2020
The WHO declared the outbreak a Public Health Emergency of International Concern
February 2020
March 2020
UN releases US$15 million for COVID-19 response
March 2020
100,000 cases of COVID-19
March 2020
The WHO characterizes COVID-19 as a pandemic
March 2020
Europe becomes epicenter of pandemic
March 2020
The US declare a state of emergency
March 2020
First COVID-19 human vaccine trials begin with Moderna mRNA vaccine
April 2020
1 million COVID-19 cases
April 2020
WHO releases guidance on mask wearing
August 2020
Lambda Variant first discovered
September 2020
Alpha Variant first discovered
September 2020
1 million COVID-19 deaths
November 2020
Pfizer and BioNTech Vaccine trials shown to be over 90% effective
November 2020
Moderna Vaccine also shown to be effective
November 2020
University of Oxford and AstraZeneca COVID-19 vaccine shown effectiveness
December 2020
Delta Variant first discovered
December 2020
WHO issues its first emergency use validation for COVID-19 vaccinations
April 2021
1 billion COVID-19 vaccine doses administered

Genome and Structure



Clinical spectrum

Asymptomatic

Symptomatic

- COUGH, SORE THROAT
- FEVER
- MUSCULOSKELETAL (ARTHRALGIA, MYALGIA)
- FATIGUE
- HEADACHE
- ANOSMIA
- DYSGEUSIA
- SEVERE DISEASE(PNEUMONIA, ARDS, MULTIORGAN FAILURE) usually associated with comorbidities

Specimen

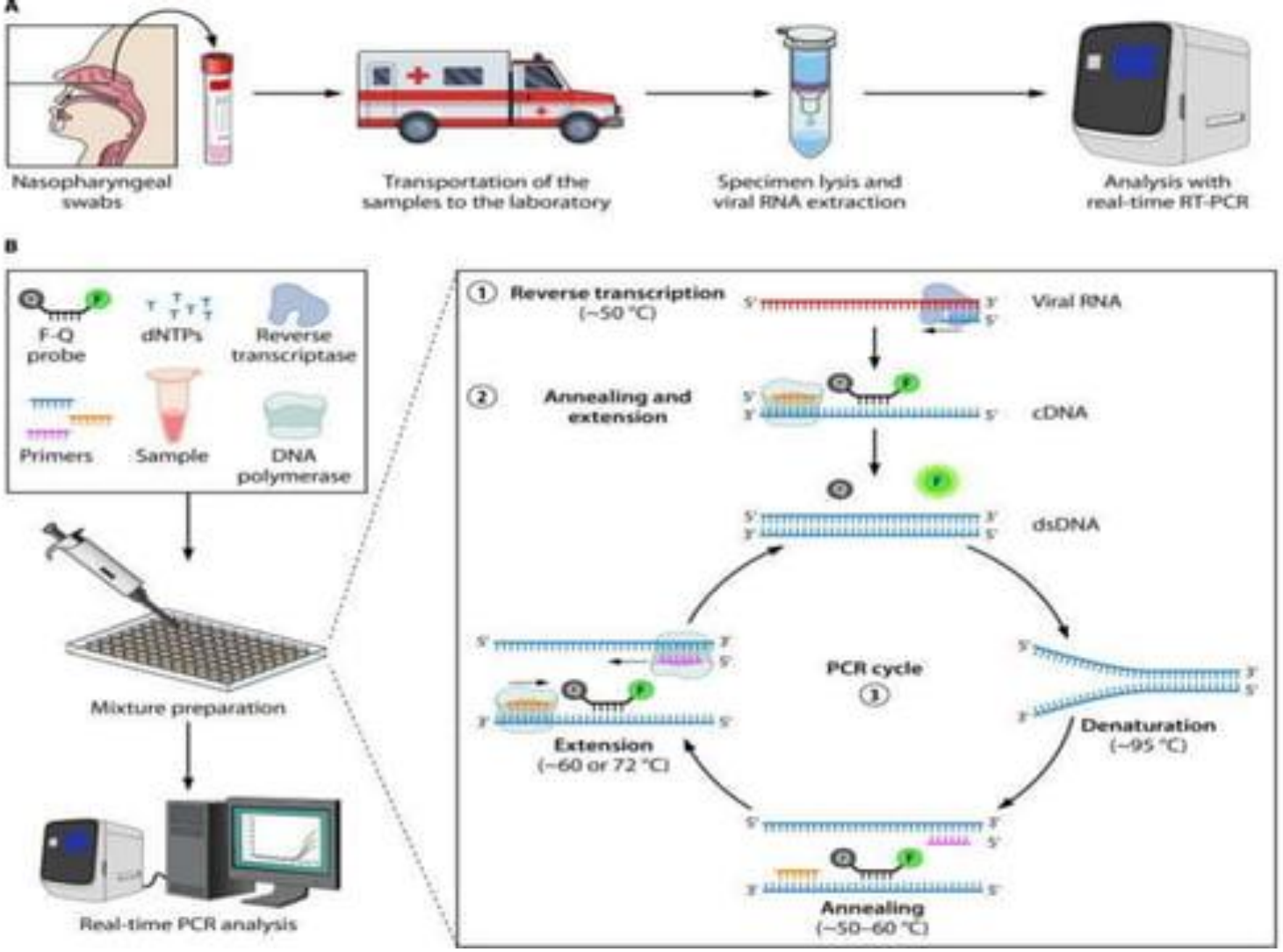
- **From the upper respiratory tract using a flocked nasopharyngeal (NP) swab placed in universal or viral transport medium are the gold standards**
- Nasal mid turbinate swabs
- Sampling of the anterior nares (Na)
- Oropharyngeal (OP) swabs
- Washes/aspirates from the nasopharynx, nose, or throat
- **Paired collection using an OP swab along with sampling of the anterior nares was shown to be equivalent to NP swab collection for the detection of SARS-CoV-2**
- Less common: saliva and throat gargles (amenable to self-collection, large-scale population-based surveillance)

Molecular methods

- Real time PCR
- RT PCR based rapid tests: Xpert Xpress SARS-CoV-2 assay on the Cepheid GeneXpert platform results in about 45 min
- Isothermal NAAT technologies: transcription-mediated amplification (TMA), nicking enzyme-assisted reaction (NEAR), loop-mediated isothermal amplification (LAMP), recombinase polymerase amplification (RPA), and systems using clustered regularly interspaced short palindromic repeat (CRISPR)–CRISPR-associated (Cas) (CRISPR-Cas) systems

Isothermal amplification technologies (IATs) are conducted at a constant temperature, eliminating the need for expensive equipment such as thermocyclers. The principles behind IATs rely on thermal or enzymatic denaturation of nucleic acids, followed by nucleic acid amplification reactions

Device/Assay	Method	Target genes	Specimen types	Time/throughput	LoD
cobas 6800/cobas SARS-CoV 2 (Roche Molecular Systems, USA)	RT-PCR	ORF1a + E	NS,NPS,OPS	3 h for the first-run results but 90 min per run in continuous mode/864 samples per 8 h	46 copies/ml
Abbott m2000/RealTime SARS-CoV-2 (Abbott Diagnostics, USA)	RT-PCR	RdRp+N	NS,NPS,OPS,BAL Fluid	7 h per run/470 samples per 24 h	100 copies/ml
FilmArray/BioFire Respiratory Panel 2.1 (BioFire Diagnostics, USA)	RT-PCR	S+M	NPS	2-min hands-on time/1 h per run	160 copies/ml
GeneXpert Xpress/Xper	RT-PCR	E+N2	NS, NPS, OPS	1-min hands-on time/45 min per	0.02 PFU/ml


A
Nasopharyngeal swabs
Transportation of the samples to the laboratory
Specimen lysis and viral RNA extraction
Analysis with real-time RT-PCR
B
F-Q probe
dNTPs
Reverse transcriptase
Primers
Sample
DNA polymerase
Mixture preparation
Real-time PCR analysis
1 Reverse transcription (~50 °C)
Viral RNA
2 Annealing and extension
cDNA
dsDNA
PCR cycle 3
Extension (~60 or 72 °C)
Denaturation (~95 °C)
Annealing (~50–60 °C)

Antigen and antibody Detection

lateral flow rapid diagnostic tests (RDTs) is often less sensitive than molecular methods but these tests can detect SARS-CoV-2 antigen reliably when the viral load is high in the clinical specimens (i.e., typically from 1 to 3 days before the onset of symptoms to 5 to 7 days after symptom onset)

Antibodies begin to appear 6 days after symptom onset, as viral RNA levels begin to decline,, the first detectable antibody in human blood is immunoglobulin M (IgM), followed by immunoglobulin G (IgG)

Viral shedding

RT-PCR false-negatives minimized by testing 2 to 3 days after symptom onset, with an average time of symptom onset of 5 days post exposure (Repeat testing can be considered for individuals with an initial negative test result but for whom there is a high level of clinical suspicion)

Median duration of viral shedding dependent on disease severity and host factors (age, immunocompromising conditions, medical comorbidities).

Individuals with mild disease clear the virus within 10 to 20 days, in some cases with severe COVID-19, the duration of shedding can be prolonged (reported to date were 83 and 111 days after symptom onset)

Biomarkers

- White blood cell (WBC) count
- Markers for inflammatory conditions (C-reactive protein [CRP], procalcitonin [PCT], or interleukin 6 [IL-6])
- Tests for anticoagulation
- Indicators of tissue damage (alanine aminotransferase [ALT], aspartate aminotransferase [AST], lactate dehydrogenase [LDH], and creatine kinase [CK]).

Most sensitive: Decrease in the lymphocyte count and increases in the inflammatory markers CRP and IL-6

Patients with critical illness have high plasma levels of inflammatory markers, and elevated levels of d-dimer and lymphopenia have been associated with an increased risk of death

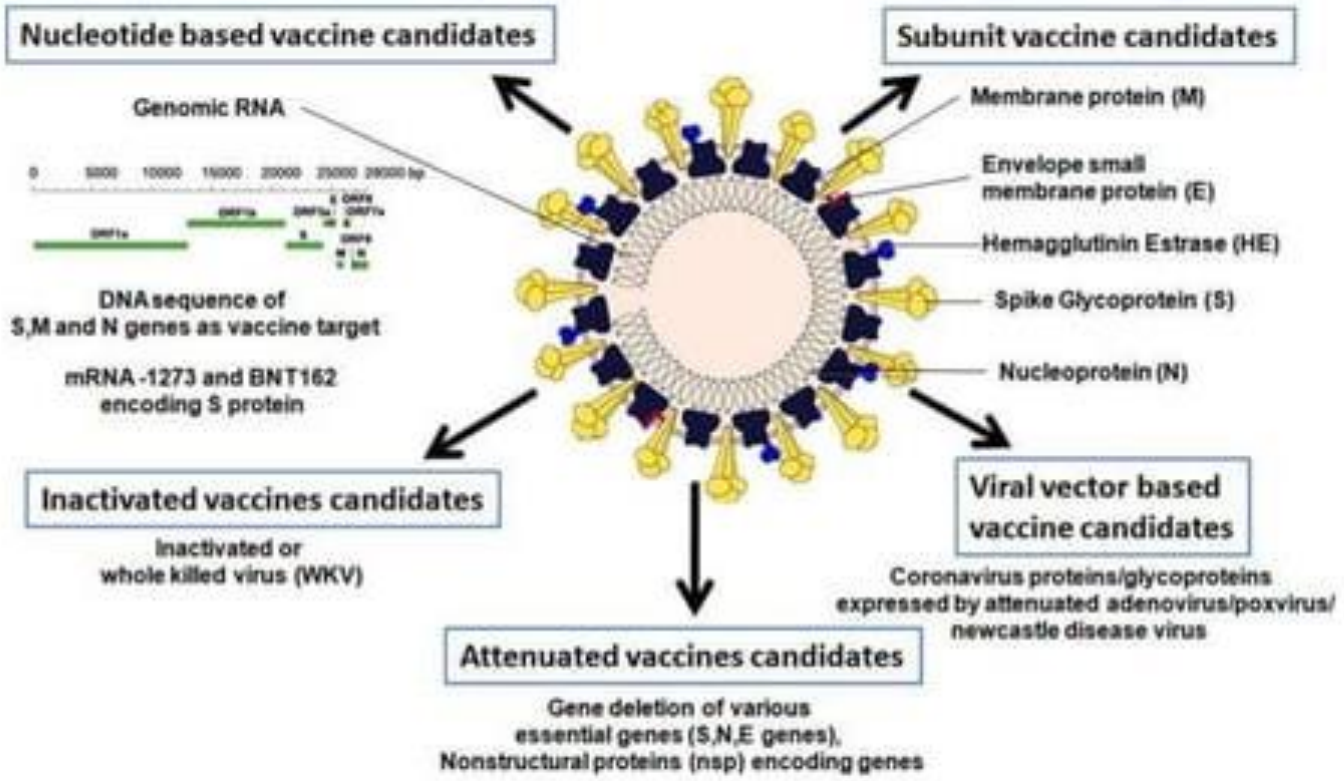
Diagnostic Imaging

- Chest X ray [CXR])
- Computed tomography (CT) scan
- Ultrasound, magnetic resonance imaging (MRI)
- Positron emission tomography-CT (PET/CT)

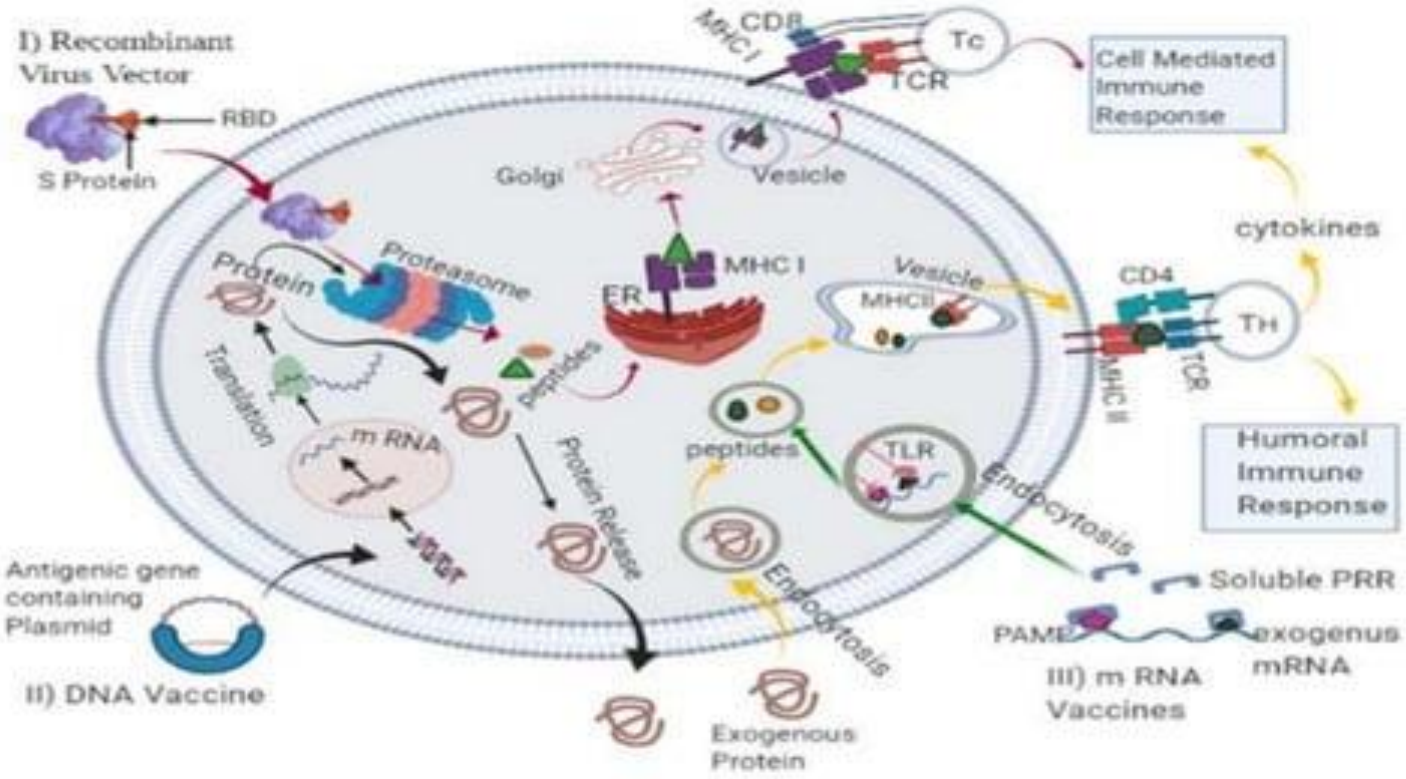
CT scans are the most frequently used methods for diagnosis of lower tract involvement or follow-up of COVID-19 cases

Typical features of a chest CT image in COVID-19 are ground-glass or reticular opacities (GGOs) with or without consolidations that present bilaterally, peripherally, or in posterior distributions

Vaccine Strategy and candidates



Mechanism of action



Mechanism of action cont..

I) **Recombinant** Virus Vector act as an endogenous antigen, thus after processing in the proteasome, they are presented by MHC I to the CD8+ Tc cells leading to **Cell Mediated Immune (CMI) response**

II) **DNA Vaccine** are transcribed and translated in the host cell, the protein synthesized then moves to MHC class I pathway or the protein would be released outside the host cell where it act as an exogenous antigen and are presented by MHC II to the CD4+ TH cells leading to **humoral immune response**, also release of some cytokines by TH cells leads to **CMI response** as well

III) **mRNA vaccines** exposed PAMP (Pathogen Associated Molecular Pattern)are recognized by the soluble PRR (Pathogen Recognition Receptor) endocytosed and lead to MHC class II pathway, Thus eliciting the **humoral immune response**.

Vaccines in India

Covaxin : Bharat Biotech developed in collaboration with the Indian Council of Medical Research (ICMR) - National Institute of Virology (NIV). This indigenous, inactivated vaccine is developed and manufactured in Bharat Biotech's BSL-3 (Bio-Safety Level 3) high containment facility.

Covishield:The Serum Institute of India (SII) . It is a recombinant, replication-deficient chimpanzee adenovirus vector encoding the SARS-CoV-2 Spike (S) glycoprotein.

Sputnik V: Dr Reddys Laboratories Limited and Sputnik LLC . Its plasmid DNA vaccine.

ZyCoV-D: Zydus Cadila, focused on discovering and developing NCEs, Novel Biologicals, Biosimilars and Vaccines.

Biological E's novel Covid-19 vaccine

Biological E. Limited is conducting a prospective open label randomised Phase-I seamlessly followed by Phase-II study to assess the safety, reactogenicity and immunogenicity of Biological E's novel Covid-19 vaccine containing Receptor Binding Domain of SARS-CoV-2 for protection against Covid-19 disease when administered intramuscularly in a two dose schedule(0, 28D) to healthy volunteers

BBV154 - Intranasal vaccine

Bharat Biotech is conducting Multicenter Study to Evaluate the Reactogenicity, Safety, and Immunogenicity of an Intranasal Adenoviral vector COVID-19 vaccine (BBV154) in Healthy Volunteers. BBV154 is an intranasal vaccine stimulates a broad immune response – neutralizing IgG, mucosal IgA, and T cell responses. Immune responses at the site of infection (in the nasal mucosa) – essential for blocking both infection and transmission of COVID-19

COVOVAX

Indian Council of Medical Research and Serum Institute of India jointly performing a phase 2/3, observer-blind, randomized, controlled study to determine the safety and immunogenicity of COVOVAX [SARS-CoV-2 recombinant spike protein nanoparticle vaccine (SARS-CoV-2 rS) with Matrix-M1™ adjuvant] in Indian adults

mRNA based vaccine (HGCO19)

Randomized, Phase I/II, Placebo-controlled, Dose-Ranging, study to evaluate the Safety, Tolerability and Immunogenicity of the candidate HGCO19 (COVID-19 vaccine) in healthy adult subjects. The trial is being conducted by Gennova Biopharmaceuticals Limited.

CORBEVAX

Corbevax is a protein subunit COVID-19 vaccine developed by Texas Children's Hospital Center for Vaccine Development and Baylor College of Medicine in Houston, Texas and Dynavax technologies based in Emeryville, California. It is licensed to Indian biopharmaceutical firm Biological E. Limited (BioE) for development and production.

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THANK YOU