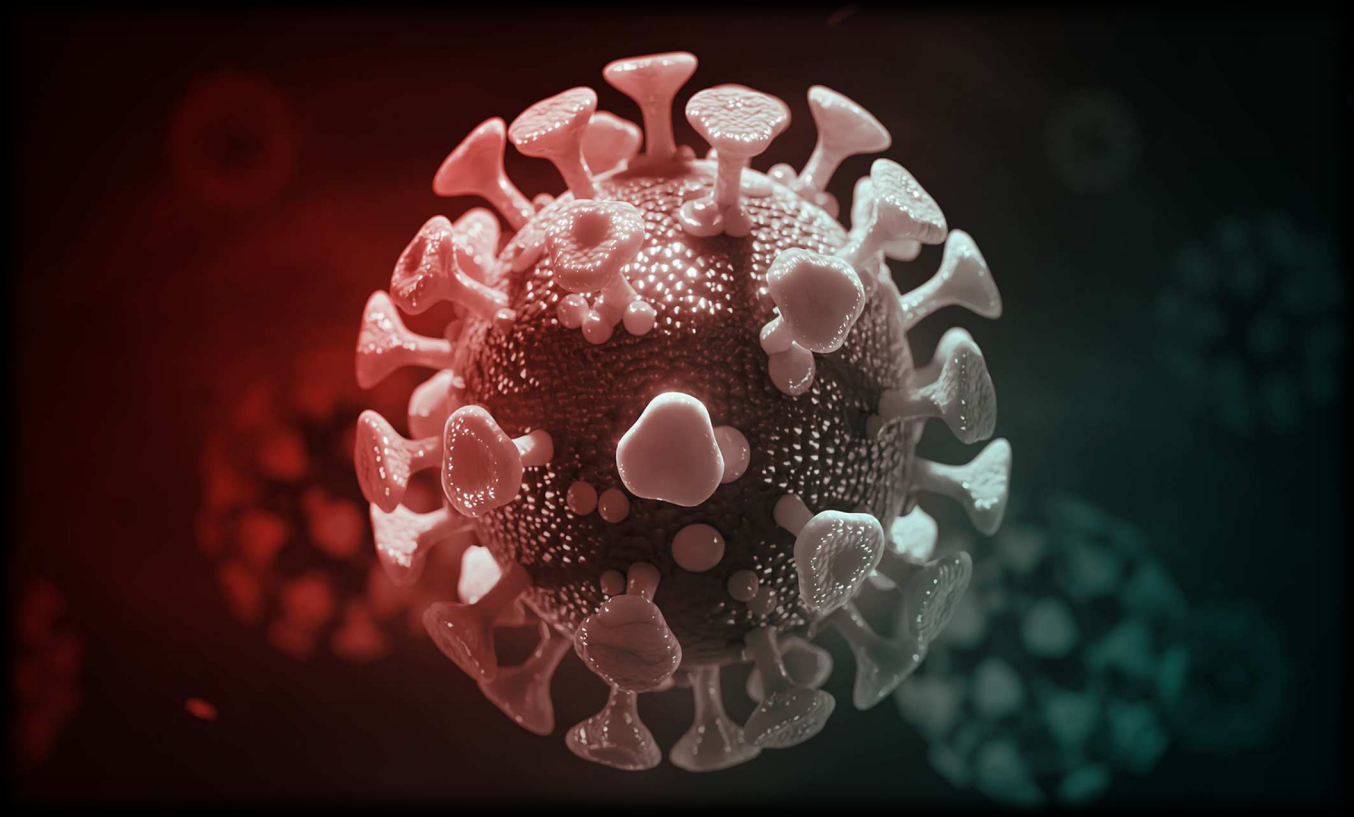
SARS-CoV-2 (COVID-19)

A COMPILATION OF DATA ON COVID-19



Guided by:

Dr. Pramod Kumar A V

Dr. Vinni T K

Dr. Swetha Ashok

Dr. Nihida Nizar

Dr. Libin C

Department of Prosthodontics

Govt. Dental College, Kozhikode

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DEPT. OF PROSTHODONTICS

GOVT.DENTAL COLLEGE, KOZHIKODE

Updated till June 2021

CONTENT

1. INTRODUCTION

Throughout recorded history, humans have been assaulted by severe epidemics of infectious disease. These epidemics have exacted a terrible toll, and some have changed the course of history. The successive waves of bubonic plague, the Black Death, killed 50% or more of European populations during the Middle Ages, and smallpox and other imported infectious diseases allowed the rapid European conquest of the Americas by virtually wiping out many native populations. Not all epidemics are restricted to specific geographical regions; those that spread worldwide are designated pandemics. And pandemics are not restricted to humans. Plant and animal virus pandemics have the potential to disrupt agriculture and the food supply. Two notable, widespread viral pandemics of the last century, the 1918 influenza and acquired immune deficiency syndrome (AIDS), had devastating consequences. Coming at the end of World War I, with millions of displaced persons, the absence of antibiotics to cure secondary bacterial infections, and insufficient healthcare resources, the 1918 influenza pandemic killed tens of millions of people. AIDS, caused by human immunodeficiency virus, was similarly lethal before being brought under relative control by highly effective antiretroviral drugs.[1]

The year 2019 saw the emergence of a new pandemic, coronavirus disease 2019 (COVID-19), caused by a previously unknown virus, severe acute respiratory syndrome coronavirus 2 (SARS- CoV-2). Although SARS-CoV-2 infection usually causes a relatively mild disease, COVID-19 can present with severe pneumonia, leading to respiratory collapse and death.[1]

The COVID – 19 pandemic has been evolving since it first surfaced in China. With more data and inputs from around the world, there is now better understanding about the disease epidemiology, transmission dynamics and treatment.[2]

Coronaviruses (CoVs) are a positive-sense single-stranded RNA viruses that cause diseases in humans and animals. The human coronaviruses (HCoVs) were first identified as causes of acute upper respiratory infection (URI) in 1962. Over the past few years, HCoVs have more often been found to be associated with severe upper and lower respiratory tract infection (RTI). They have been identified as a main cause of pneumonia in older adults and immunocompromised patients. Over the last two decades, two highly pathogenic human coronaviruses were identified, including coronaviruses associated with severe acute respiratory

syndrome (SARS-CoV-2) and the Middle East respiratory syndrome (MERS-CoV) which emerged in different regions of the world. On December 31, 2019, a new strain of coronavirus was isolated and named as severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) by the International Committee on Taxonomy of Viruses (ICTV) from patients with pneumonia of unknown etiology in Wuhan city, China. On March 11, 2020, the World Health Organization (WHO) announced that COVID-19 is a 'public-health emergency of international concern'.[3]

In the last two decades, SARS-CoV and MERS-CoV have caused epidemics with mortality rates of approximately 9.5% and 34.4%, respectively. *COVID-19* was the third highly epidemic disease detected, with a lower mortality rate than SARS and MERS, different from country to country. The higher transmissibility, varied clinical manifestations, and lower pathogenicity of COVID- 19 could be due to diversity in the biology and genome structure of the SARS-CoV-2 compared to SARS-CoV and MERS-CoV.[4]

The current management of COVID-19 is based generally on supportive therapy and treatment to prevent respiratory failure. The effective option of antiviral therapy and vaccination is currently under evaluation and development. An exit strategy for a path back to normal life is required, which should involve a multi-prong effort towards development of new treatment and vaccine to protect public health worldwide and future COVID-19 outbreaks.[3]

Considering the high rate of transmissibility along with its mysterious pathophysiology in the immune naive population infected with SARS-CoV-2, it was of paramount importance to develop robust diagnostic tools for detection and formulation of treatment protocol. Moreover, as the symptoms of COVID -19 can mimic other respiratory viral infections, thus the widespread testing capacity building for SARS-CoV-2 detection and diagnosis has been playing a pivotal role in identifying and isolating the infected persons and thereby curbing the spread of the virus since the time COVID 19 has been declared as a Pandemic.[5]

2. STRUCTURE OF CORONA VIRUS AND ITS VARIANTS

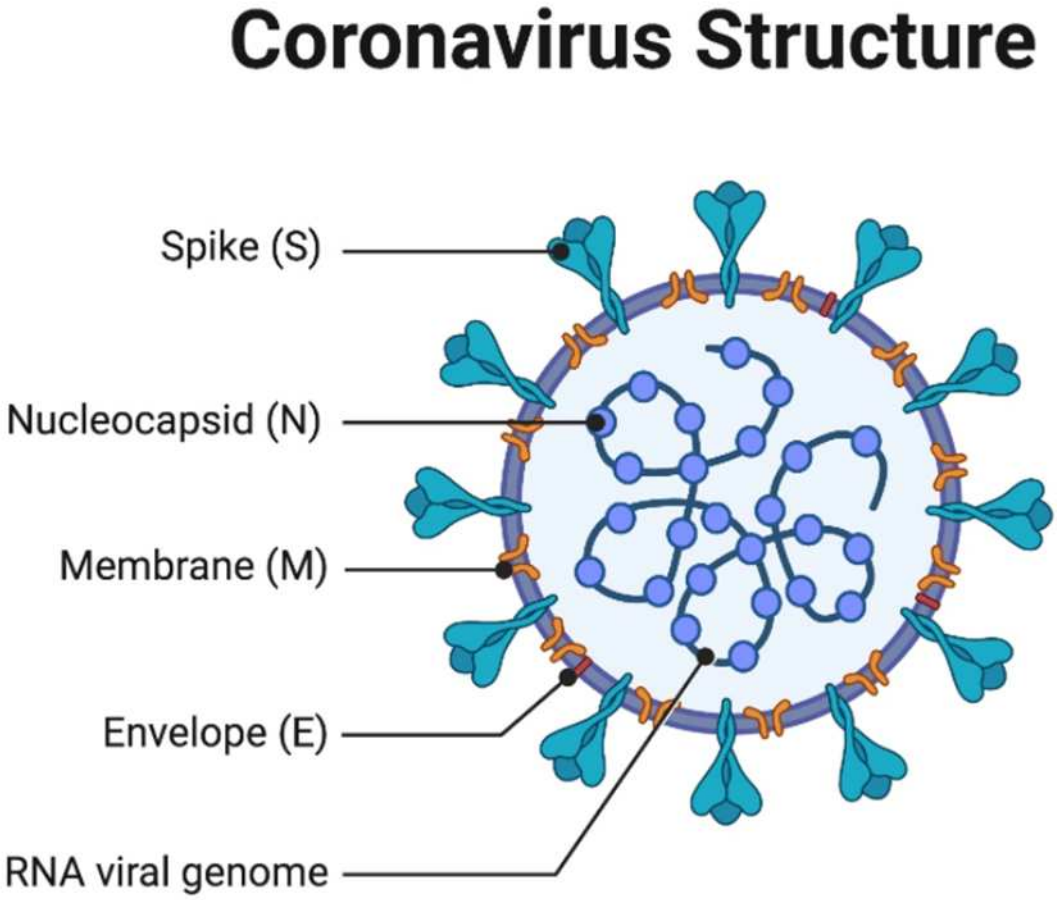
All viruses are parasites which can only reproduce within cells. Thus, they are very different from bacteria and fungi, which are self-reproducing, often in soil, water, organic wastes, sewage, or within organisms.[6]

Animal and plant viruses fall into two general classes, those in which the genetic material is long DNA molecules, and those in which the genetic material is RNA molecules. Among the DNA viruses are Herpes, Adenoviruses, and wart viruses. Coronaviruses, named for their "sun-like" shape observed in the electron microscope, use RNA molecules to encode their genes, as do influenza viruses, HIV, and rhinoviruses (common cold). SARS-CoV-2, the virus that causes COVID-19, infects mammals and birds. It is closely related to the viruses causing the earlier SARS (Severe Acute Respiratory Syndrome) and MERS (Middle East Respiratory Syndrome) outbreaks.[6]

Coronaviruses (CoVs) are an important cause of illness in humans and animals. Most human coronaviruses commonly cause relatively mild respiratory illnesses. Coronavirinae, a subfamily of RNA viruses consisting of four genera, namely Alphacoronaviruses (alpha-CoV), Beta coronaviruses (beta-CoV), Gamma coronaviruses (gamma-CoV), and Delta coronaviruses (delta-CoV). Alpha-CoV and beta-CoV are able to infect humans, usually resulting in respiratory illness. Beta-CoV comprise the three most pathogenic coronaviruses known to date: the severe acute respiratory syndrome virus (SARS-CoV), the Middle East respiratory syndrome virus (MERS-CoV), and the SARS-CoV-2 virus, responsible for the currently ongoing COVID-19 pandemic . Coronaviruses (CoV) are characterized by the largest known positive-sense single stranded RNA genomes (~26–32 kb), with a highly conserved architecture. Despite the relatively poor fidelity of viral RNA-dependent RNA polymerases (RdRp), overall long-term integrity of such large genomes is ensured by the presence of the 30 –50 exonuclease ExoN domain of the non-structural protein nsp14, that enables proof-reading at genome replication.[7]

Structure of corona virus

The coronavirus particles are organized with long RNA polymers tightly packed into the centre of the particle, and surrounded by a protective capsid, which is a lattice of repeated protein molecules referred to as coat or capsid proteins. In coronavirus, these proteins are called nucleocapsid (N). The coronavirus core particle is further surrounded by an outer membrane envelope made of lipids (fats) with proteins inserted. These membranes derive from the cells in which the virus was last assembled but are modified to contain specific viral proteins, including the spike (S), membrane (M), and envelope (E) proteins.[8]



Picture courtesy: Biorender, Jonathan King

A key set of the proteins in the outer membrane project out from the particle and are known as spike proteins (S). It is these proteins which are recognized by receptor proteins on the host cells which will be infected.

The viral envelope is made up of a lipid bilayer in which the membrane (M), envelope (E) and spike (S) structural proteins are anchored. The molar ratio of E:S:M in the lipid bilayer is approximately 1:20:300. The E and M protein are the structural proteins that combined with the lipid bilayer to shape the viral envelope and maintain its size. S proteins are needed for interaction with the host cells. But human coronavirus NL63 is peculiar in that its M protein has the binding site for the host cell, and not its S protein. The diameter of the envelope is 85 nm. The envelope of the virus in electron micrographs appears as a distinct pair of electron-dense shells (shells that are relatively opaque to the electron beam used to scan the virus particle).[8]

M protein

The M protein is the main structural protein of the envelope that provides the overall shape and is a type III membrane protein. It consists of 218 to 263 amino acid residues and forms a layer 7.8 nm thick. It has three domains, a short N-terminal ectodomain, a triple-spanning transmembrane domain, and a C-terminal endodomain. The C-terminal domain forms a matrix-like lattice that adds to the extra-thickness of the envelope. Different species can have either N- or O-linked glycans in their protein amino-terminal domain. The M protein is crucial during the assembly, budding, envelope formation, and pathogenesis stages of the virus lifecycle.[6]

E protein

The E proteins are minor structural proteins and highly variable in different species. There are only about 20 copies of the E protein molecule in a coronavirus particle. They are 8.4 to 12 kDa in size and are composed of 76 to 109 amino acids. They are integral proteins (i.e. embedded in the lipid layer) and have two domains namely a transmembrane domain and an extra membrane C-terminal domain. They are almost fully α-helical, with a single α-helical transmembrane domain, and form pentameric (five-molecular) ion channels in the lipid bilayer. They are responsible for virion assembly, intracellular trafficking and morphogenesis (budding).[7]

S protein

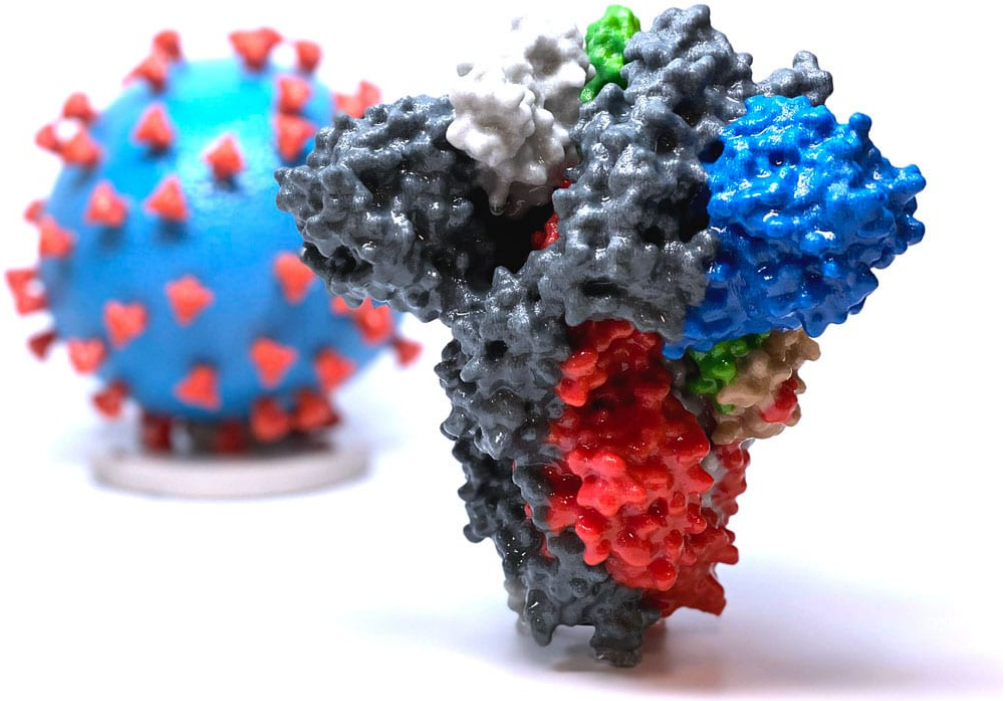
The spikes are the most distinguishing feature of coronaviruses and are responsible for the corona- or halo-like surface. On average a coronavirus particle has 74 surface spikes. Each

spike is about 20 nm long and is composed of a trimer of the S protein. The S protein is in turn composed of an S1 and S2 subunit. The homotrimeric S protein is a class I fusion protein which mediates the receptor binding and membrane fusion between the virus and host cell. The S1 subunit forms the head of the spike and has the receptor-binding domain (RBD). The S2 subunit forms the stem which anchors the spike in the viral envelope and on protease activation enables fusion. The two subunits remain noncovalently linked as they are exposed on the viral surface until they attach to the host cell membrane. In a functionally active state, three S1 are attached to two S2 subunits. The subunit complex is split into individual subunits when the virus binds and fuses with the host cell under the action of proteases such as cathepsin family and transmembrane protease serine 2 (TMPRSS2) of the host cell.[9]

S1 proteins are the most critical components in terms of infection. They are also the most variable components as they are responsible for host cell specificity. They possess two major domains named N-terminal domain (S1-NTD) and C-terminal domain (S1-CTD), both of which serve as the receptor-binding domains. The NTDs recognize and bind sugars on the surface of the host cell. An exception is the MHV NTD that binds to a protein receptor carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1). S1-CTDs are responsible for recognizing different protein receptors such as angiotensin-converting enzyme 2 (ACE2), aminopeptidase N (APN), and dipeptidyl peptidase 4 (DPP4).[9]

A subset of coronaviruses (specifically the members of beta coronavirus subgroup A) also has a shorter spike-like surface protein called hemagglutinin esterase (HE).The HE proteins occur as homodimers composed of about 400 amino acid residues and are 40 to 50 kDa in size. They appear as tiny surface projections of 5 to 7 nm long embedded in between the spikes. They help in the attachment to and detachment from the host cell.[9]

Inside the envelope, there is the nucleocapsid, which is formed from multiple copies of the nucleocapsid (N) protein, which are bound to the positive-sense single-stranded RNA genome in a continuous beads-on-a-string type conformation. N protein is a phosphoprotein of 43 to 50 kDa in size, and is divided into three conserved domains. The majority of the protein is made up of domains 1 and 2, which are typically rich in arginines and lysines. Domain 3 has short carboxy terminal end and has a net negative charge due to excess of acidic over basic amino acid residues.[10]



Courtesy: Global Biodefense

RNA Viral Genome

The genomic RNA (gRNA) has a 50 cap and 30 polyA tail, allowing direct translation of the non-structural polyproteins (nsp) ORF1a, and ORF1b, followed by assembly of the replicase-transcriptase complex (RTC). The RTC drives both genome replication and discontinuous transcription of subgenomic mRNAs (sgRNAs). Discontinuous transcription is mediated by short AU-rich transcription regulating sequences (TRSs), located either right downstream of the 50 leader (TRS-L), or right upstream of each viral ORF (TRS-B), except for ORF1a and ORF1b. The resulting sgRNAs are further translated to produce the structural proteins spike (S), envelope (E), membrane (M) and nucleocapsid (N), as well as several accessory proteins.[11]

SARS-CoV-2 Variant Classifications [12]

Genetic variants of SARS-CoV-2 have been emerging and circulating around the world throughout the COVID-19 pandemic. Viral mutations and variants in the United States are routinely monitored through sequence-based surveillance, laboratory studies, and epidemiological investigations.

A U.S government SARS-CoV-2 Interagency Group (SIG) developed a Variant Classification scheme that defines three classes of SARS-CoV-2 variants:

a. Variant of Interest
b. Variant of Concern
c. Variant of High Consequence

The B.1.1.7 (Alpha), B.1.351 (Beta), B.1.617.2 (Delta), and P.1 (Gamma) variants circulating in the United States are classified as variants of concern. To date, no variants of high consequence have been identified in the United States. Laboratory studies suggest specific monoclonal antibody treatments may be less effective for treating cases of COVID-19 caused by variants with certain substitutions or combinations of substitutions in the spike protein.

L452R is present in B.1.526 (Iota), B.1.427 (Epsilon), and B.1.429 (Epsilon) lineages, as well as the B.1.617 (Kappa, Delta) lineages and sub-lineages. E484K is present in B.1.525 (Eta), P.2 (Zeta), P.1 (Gamma), and B.1.351 (Beta), but only some strains of B.1.526 (Iota) and B.1.1.7 (Alpha). The combination of K417N, E484K, and N501Y substitutions is present in B.1.351 (Beta). The combination of K417T, E484K, and N501Y substitutions is present in P.1 (Gamma). P.2 (Zeta) has been removed from the variants of interest list due to declining prevalence and very few detections in recent months. In addition to variants of interest and variants of concern, CDC continues to monitor all variants circulating within the United States.

a. Variant of Interest

A variant with specific genetic markers that have been associated with changes to receptor binding, reduced neutralization by antibodies generated against previous infection or vaccination, reduced efficacy of treatments, potential diagnostic impact, or predicted increase in transmissibility or disease severity.

Possible attributes of a variant of interest:

i. Specific genetic markers that are predicted to affect transmission, diagnostics, therapeutics, or immune escape.
ii. Evidence that it is the cause of an increased proportion of cases or unique outbreak clusters.
iii. Limited prevalence or expansion in the US or in other countries.

A variant of interest might require one or more appropriate public health actions, including enhanced sequence surveillance, enhanced laboratory characterization, or epidemiological investigations to assess how easily the virus spreads to others, the severity of disease, the efficacy of therapeutics and whether currently authorized vaccines offer protection.

Current variants of interest in the United States that are being monitored and characterized are listed below.

Selected Characteristics of SARS-CoV-2 Variants of Interest

B.1.427

Spike Protein Substitutions: L452R, D614G

Name : 20C/S:452R

WHO Label: Epsilon

First Identified: United States-(California)

Attributes:

i. ~20% increased transmission
ii. Modest decrease in susceptibility to the combination of bamlanivimab and etesevimab; however, the clinical implications of this decrease are not known. Alternative monoclonal antibody treatments are available.
iii. Reduced neutralization by convalescent and post-vaccination sera.

iv. Deescalated from a VOC on June 29, 2021, due to the significant decrease in the proportion of B.1.427 lineage viruses circulating nationally and available data indicating that vaccines and treatments are effective against this variant.

B.1.429

Spike Protein Substitutions: S13I, W152C, L452R, D614G

Name : 20C/S:452R

WHO Label: Epsilon

First Identified: United States-(California)

Attributes:

i. ~20% increased transmission
ii. Reduced susceptibility to the combination of bamlanivimab and etesevimab; however, the clinical implications of this decrease are not known. Alternative monoclonal antibody treatments are available.
iii. Reduced neutralization by convalescent and post-vaccination sera.
iv. Deescalated from a VOC on June 29, 2021, due to the significant decrease in the proportion of B.1.429 lineage viruses circulating nationally and available data indicating that vaccines and treatments are effective against this variant.

B.1.525

Spike Protein Substitutions: A67V, 69del, 70del, 144del, E484K, D614G, Q677H, F888L

Name: 20A/S:484K

WHO Label: Eta

First Identified: United Kingdom/Nigeria – December 2020

Attributes:

i. Potential reduction in neutralization by some Emergency Use Authorization (EUA) monoclonal antibody treatments
ii. Potential reduction in neutralization by convalescent and post-vaccination sera

B.1.526

Spike Protein Substitutions: L5F, (D80G*), T95I, (Y144-*), (F157S*), D253G, (L452R*), (S477N*), E484K, D614G, A701V, (T859N*), (D950H*), (Q957R*)

Name : 20C/S:484K

WHO Label: Iota

First Identified: United States (New York) – November 2020

Attributes:

i. Reduced susceptibility to the combination of bamlanivimab and etesevimab monoclonal antibody treatment; however, the clinical implications of this are not known. Alternative monoclonal antibody treatments are available.
ii. Reduced neutralization by convalescent and post-vaccination sera
iii. B.1.526.1 sublineage has been consolidated with this parent lineage

B.1.617.1

Spike Protein Substitutions: (T95I), G142D, E154K, L452R, E484Q, D614G, P681R, Q1071H

Name : 20A/S:154K

WHO Label: Kappa

First Identified: India – December 2020

Attributes:

i. Potential reduction in neutralization by some EUA monoclonal antibody treatments
ii. Potential reduction in neutralization by post-vaccination sera

B.1.617.3

Spike Protein Substitutions: T19R, G142D, L452R, E484Q, D614G, P681R, D950N

Name : 20A

First Identified: India – October 2020

Attributes:

i. Potential reduction in neutralization by some EUA monoclonal antibody treatments
ii. Potential reduction in neutralization by post-vaccination sera

b.Variant of Concern

A variant for which there is evidence of an increase in transmissibility, more severe disease (e.g., increased hospitalizations or deaths), significant reduction in neutralization by antibodies generated during previous infection or vaccination, reduced effectiveness of treatments or vaccines, or diagnostic detection failures**.**

Possible attributes of a variant of concern:

In addition to the possible attributes of a variant of interest

- Evidence of impact on diagnostics, treatments, or vaccines
    i. Widespread interference with diagnostic test targets
    ii. Evidence of substantially decreased susceptibility to one or more class of therapies

iii. Evidence of significant decreased neutralization by antibodies generated during previous infection or vaccination
iv. Evidence of reduced vaccine-induced protection from severe disease
v. Evidence of increased transmissibility
vi. Evidence of increased disease severity

Selected Characteristics of SARS-CoV-2 Variants of Concern

B.1.1.7

Spike Protein Substitutions: 69del, 70del, 144del, (E484K*), (S494P*), N501Y, A570D, D614G, P681H, T716I, S982A, D1118H (K1191N*)

Name : 20I/501Y.V1

WHO Label*:* Alpha

First Identified: United Kingdom

Attributes:

i. ~50% increased transmission
ii. Potential increased severity based on hospitalizations and case fatality rates
iii. No impact on susceptibility to EUA monoclonal antibody treatments
iv. Minimal impact on neutralization by convalescent and post-vaccination sera

B.1.351

Spike Protein Substitutions: D80A, D215G, 241del, 242del, 243del, K417N, E484K, N501Y, D614G, A701V

Name : 20H/501.V2

WHO Label: Beta

First Identified: South Africa

Attributes:

i. ~50% increased transmission
ii. Significantly reduced susceptibility to the combination of bamlanivimab and etesevimab monoclonal antibody treatment,[7] but other EUA monoclonal antibody treatments are available
iii. Reduced neutralization by convalescent and post-vaccination sera

B.1.617.2

Spike Protein Substitutions: T19R, (V70F*), T95I, G142D, E156-, F157-, R158G, (A222V*), (W258L*), (K417N*), L452R, T478K, D614G, P681R, D950N

Name : 21A/S:478K

WHO Label: Delta

First Identified: India

Attributes:

i. Increased transmissibility
ii. Potential reduction in neutralization by some EUA monoclonal antibody treatments
iii. Potential reduction in neutralization by post-vaccination sera
iv. AY.1, AY.2 and AY.3 are currently aggregated with B.1.617.2. As data are available, CDC will continue to evaluate the independent classification of AY.1, AY.2, and AY.3.

P.1

Spike Protein Substitutions: L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I

Name : 20J/501Y.V3

WHO Label: Gamma

First Identified: Japan/Brazil

Attributes:

i. Significantly reduced susceptibility to the combination of bamlanivimab and etesevimab monoclonal antibody treatment,[7] but other EUA monoclonal antibody treatments are available
ii. Reduced neutralization by convalescent and post-vaccination sera

c.Variant of High Consequence

A variant of high consequence has clear evidence that prevention measures or medical countermeasures (MCMs) have significantly reduced effectiveness relative to previously circulating variants.

Possible attributes of a variant of high consequence:

In addition to the possible attributes of a variant of concern

Impact on Medical Countermeasures (MCM)

i. Demonstrated failure of diagnostics
ii. Evidence to suggest a significantly reduction in vaccine effectiveness, a disproportionately high number of vaccine breakthrough cases, or very low vaccine-induced protection against severe disease
iii. Significantly reduced susceptibility to multiple Emergency Use Authorization (EUA) or approved therapeutics
iv. More severe clinical disease and increased hospitalizations

3. SIGNS AND SYMPTOMS

The clinical features of COVID-19 are varied and nonspecific; disease presentation can range from asymptomatic to severe pneumonia and death.[2] also the symptoms of COVID -19 can mimic other respiratory viral infections.

COVID-19 patients reporting to various Covid treatment facilities have reported the following signs and symptoms:

i. Fever,
ii. cough,
iii. general weakness/ fatigue,
iv. headache,
v. myalgia,
vi. sore throat, coryza,
vii. dyspnoea,
viii. anorexia/nausea/vomiting, diarrhoea,
ix. altered mental status.
x. Loss of smell (anosmia) or loss of taste (ageusia) preceding the onset of respiratory symptoms has also been reported. Loss of smell has been shown to increase the pre-test probability of presence of SARS-COV-2.

Older people and immune-suppressed patients in particular may present with atypical symptoms such as fatigue, reduced alertness, reduced mobility, diarrhoea, loss of appetite, delirium, and absence of fever. Children might not have fever or cough as frequently as adults.

The major risk factors for severe disease are:

i. Age more than 60 years
ii. Underlying non-Communicable diseases like cardiovascular disease, hypertension, and CAD, DM (Diabetes Mellitus) and other immunocompromised states, Chronic lung/kidney/liver disease, Cerebrovascular diseases and Obesity.[13]

Clinical Categorization Based on Symptomatology (as given in COVID-19: Treatment Guidelines for Kerala state Version 3, 25th April 2021)[14]

A	Mild sore throat / cough / rhinitis /diarrhea/anosmia
B	Fever and/or severe sore throat / cough /diarrhea/anosmia OR Category-A with any one of • Lung/ heart / liver/ kidney / neurological disease/ Hypertension / haematological disorders/ uncontrolled diabetes/ cancer /HIV- AIDS/ Cardiovascular disease • On long term steroids /immunosuppressive drugs. • Pregnant lady • Age –more than 60 years.
C	Breathlessness, chest pain, drowsiness, fall in blood pressure, haemoptysis, cyanosis [red flag signs] • Children with ILI (influenza like illness) with red flag signs (Somnolence, high/persistent fever, inability to feed well, convulsions, dyspnoea /respiratory distress, etc). • Worsening of underlying chronic conditions.

Categorization should be reassessed every 24-48 hours for Category A & B

Yuki et al. provided a classification of COVID-19 patients based on clinical features/lab investigation.[15]

Classifications	**Clinical Features/laboratory investigation**
Asymptomatic	COVID-19 nucleic acid test positive. No clinical symptoms and signs. Chest imaging – normal.
Mild	Symptoms of acute upper respiratory tract infection: fever, fatigue, myalgia, cough, sore throat, runny nose, sneezing) or digestive symptoms (nausea, vomiting, abdominal pain, diarrhoea
Moderate	Pneumonia (frequent fever, cough) with no obvious hypoxemia. Chest CT with lesions.
Severe	Pneumonia with hypoxemia (SpO2 < 92%)
Critical	Acute respiratory distress syndrome (ARDS), may have shock, encephalopathy, myocardial injury, heart failure, coagulation dysfunction and acute kidney injury

The symptoms have been reported to appear after an incubation period between 2–14 days . The period from the onset of SARS-CoV-2 symptoms to death ranged from 6 to 41 days which is dependent on the age and the status of the patient's immune system. The age range affected was mostly middle-aged patients with a mean age range of 40–59 years and older (> 60 years).

Additionally, studies reported that SARS-CoV-2 disease progressed faster among the elderly compared with those under the age of 60 years . Fever and cough are the most dominant symptoms associated with SAR-CoV-2 and the temperature range is within 39°C. About 80% of confirmed SARS- CoV-2 cases have suffered from only mild to moderate forms of the disease, with approximately 12% of patients being elderly. Asymptomatic carriers of SARS-CoV-2, who presented with a history of underlying health conditions such as hyper- tension, chronic obstructive pulmonary disease, diabetes, cardiovascular disease, have later developed critical illnesses, which manifested as respiratory failure, septic shock, multiple organ failure and eventually death.[3]

Infections in Covid patients

a. Bacterial infections

Secondary infections in COVID-19 patients are known to be associated with negative health outcomes. As per recent studies, bacterial co-infection upon admission has been reported in 3.1–3.5% of COVID-19 patients, while secondary bacterial infections, following hospitalization, occurred in up to 15% of patients. Higher risk of mortality in COVID-19 patients with bacterial super-infection has been previously reported, and several recommendations encourage empirical use of antibiotics in severely ill patients.[16]

A study by Camille et al showed that Among the 197 ICU COVID-19 patients, 77 (39.1%) had at least one bacterial pneumonia. Inaugural episodes were: 5 community-acquired pneumonia (6.5%), 7 non-ventilated hospital acquired pneumonia (9.1%) and 65 VAP (84.4%). This represents 5/77 (6.5%) pneumonia co-infections (present since admission) and 72/77 (93.5%) pneumonia super-infection (acquired during hospitalisation). The most frequent bacteria involved in Ventilator Acquired Pneumonia were *Staphylococcus aureus*(17/65, 26.2%), *Pseudomonas aeruginosa* (11/65, 16.9%), *Klebsiella pneumoniae* (9/65, 13.8%) and *Escherichia coli* (8/65, 12.3%). No difference was observed in the distribution of bacteria between early- and late-onset VAP (data not shown). Among Enterobacter ales, 11/37 *(*29.7%) were resistant to third-generation cephalosporins but susceptible to carbapenems (8 extended-spectrum beta-lactamase and 3 high-level expression of cephalosporinase) and 5/37 (13.5%) were resistant to carbapenems.[17]

b. Fungal infections

In general, there are various types of fungal infections such as candida, aspergillosis, cryptococcus, histoplasmosis and coccidioidomycosis. Mucormycosis, candida and aspergillosis are the ones observed more in those with low immunity.

i. Mucormycosis in Corona patients

Mucormycosis or zygomycosis, also called phycomycosis, initially described in 1885 by Paltauf, is an uncommon and aggressive fungal infection that usually affects patients with alteration of their immunological system. It is a lethal fungal disease, with rhinocerebral presentation being its most common form.[18]

Mucormycosis infection of the sinuses is a form of life-threatening invasive fungal sinusitis that typically affects immunocompromised individuals with an impaired neutro-philic response. Patients can include those with uncontrolled diabetes mellitus, acquired immunodeficiency syndrome, iatrogenic immunosuppression and haematological malignancies, and those who have undergone organ transplantation.[18]

A complex interplay of factors that include diabetes mellitus, any previous respiratory pathology, immunosuppressive therapy, nosocomial infection sources and systemic immune alterations of Covid-19 infection itself may lead to secondary infections, which are increasingly being recognized in view of their impact on morbidity and mortality. Furthermore, as Covid-19 is a life-threatening, infectious disease, affected patients show an overexpression of inflammatory cytokines, and impaired cell-mediated immunity with decreased cluster of differentiation 4 and 8 positive T-helper (CD4+ T and CD8+ T) cell counts, indicating susceptibility to fungal co-infections. Critically ill patients, especially those admitted to intensive care units and those who required mechanical ventilation, or who had a longer duration of hospital stays, even as long as 50 days, were more likely to develop fungal co-infections.[18]

Signs and symptoms

Mucormycosis is characterized by the presence of hyphal invasion of sinus tissue and a time course of less than four weeks. Clinically, rhinocerebral mucormycosis can present with atypical signs and symptoms similar to complicated sinusitis, such as *nasal blockade, crusting, proptosis, facial pain and oedema, ptosis, chemosis, and even ophthalmoplegia, with headache and fever* and various neurological signs and symptoms of intracranial palate region but is not characteristic.

A black eschar is often seen in the nasal cavity or over the hard extension is present. Histological features include mycotic infiltration of blood vessels, vasculitis with thrombosis, tissue infarction, hemorrhage, and acute neutrophilic infiltrate. Without early diagnosis and treatment, there may be rapid progression of the disease, with reported mortality rates from intra-orbital and intracranial complications of 50–80 percent. Even with prompt diagnosis, treatment of underlying diseases, and aggressive medical and surgical intervention, the management is often not effective, leading to an extension of the infection and ultimately death. Recently, a change in the incidence of mucormycosis infection of the sinuses has been observed, with more cases being diagnosed much more frequently. Over the past few months, there has been a sudden rise in cases of invasive fungal sinusitis, especially mucormycosis.

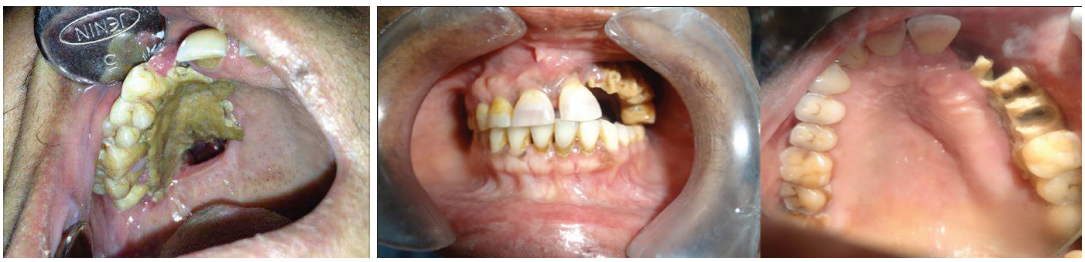
Extensive use of steroids in Covid-19 management can also suppress immunity, allowing opportunistic fungal infections to colonize. Hence, it is important to be aware that Covid-19 patients can develop further fungal infections during the middle and latter stages of this disease, especially severely ill individuals.

Mucor is a saprophytic fungus; its spores exist widely in nature, and are spread in soil, air, food and decaying organic material. Because of the low virulence potential, it may be present in the nasal mucosa of healthy people as a commensal. If the patient becomes immunosuppressed, this fungus may germinate within the paranasal sinuses, and spread intracranially or to other nearby structures such as the orbit.[18]

Signs and Symptoms dentists has to be aware of;

Discoloration of oral tissue, tongue and gums, stuffy nose, severe pain, swelling of the face and heaviness in the region below the eyes or discomfort, headache, and fever.

Also, numbness in the infraorbital, upper lip and palatal region. Unexplained toothache not relieved by medication and even root canal treatment. Tooth mobility, which is horizontal as well as vertical mobility without significant bone loss. Pus discharge from intraoral sinuses with no periapical or periodontal lesions but with associated tooth mobility. Palatal necrosis which starts from midline starting with discoloration, swelling and necrosis. Presence of non-healing extraction sockets with non-vital bone. Oro antral communication and trismus is present in some cases. Patient also reports with facial swelling which can also be in the form of periorbital swelling.[18]



Picture courtesy: Mucormycosis, aka Tthe Black Fungus"- A Dentist's Guide; Dentowesome

Diagnosis

Non-contrast computed tomography of the paranasal sinuses is usually the first investigation of choice, with gadolinium-enhanced magnetic resonance imaging being resorted to if intra-orbital or intracranial extension is suspected. Focal bony erosions and extrasinus spread are strongly suggestive of the diagnosis.

Treatment

Surgical debridement of the infected area should be performed as soon as possible once the diagnosis is confirmed. Surgery alone has been reported not to be curative, but an aggressive surgical approach has been shown to improve survival.

Amphotericin-B deoxycholate remains the anti-fungal treatment of choice to start, with its liposomal preparations preferred because of decreased nephrotoxicity. In cases refractory or

intolerant to amphotericin therapy, Posaconazole is considered a suitable alternative. Prognosis remains poor even with aggressive surgery and intra venous antifungal therapy, with reported mortality rates of 33.3–80 per cent, going up to 100 per cent in disseminated infections.[18]

ii. Candidiasis

Candida auris is an emerging fungus that presents a serious global health threat. CDC is concerned about C. auris for three main reasons:

a. It is often multidrug-resistant, meaning that it is resistant to multiple antifungal drugs commonly used to treat Candida infections. Some strains are resistant to all three available classes of antifungals.

b. It is difficult to identify with standard laboratory methods, and it can be misidentified in labs without specific technology. Misidentification may lead to inappropriate management.

c. It has caused outbreaks in healthcare settings. For this reason, it is important to quickly identify C. auris in a hospitalized patient so that healthcare facilities can take special precautions to stop its spread.

In a study published in June, Chowdhary and Sharma point out that COVID-19 patients who are pre-colonized with C. auris and who require indwelling catheters have higher risks of C. auris–related bloodstream and urinary tract infections. It's also important to emphasize, they say, that COVID-19 patients in the ICU tend to share risk factors, medications, and underlying comorbidities with C. auris infections, such as diabetes, chronic kidney disease, intubation, and administration of broad-spectrum antibiotics.[19]

Limited diagnostic capabilities in developing countries may mean under-recognition of fungal coinfections in patients with COVID-19. Chowdhary and Sharma called C. auris a "lurking scourge." They warned the global medical community about the potential of C. auris as a confounding factor in COVID-19. They suggested that COVID-19 patient mortality might already have contributions from C. auris or other coinfections. A follow-up study, published in October, bore out their prediction.

Chowdhary et al1 say the patients in that study were probably infected while hospitalized, highlighting the fact that C. auris can be transmitted in healthcare settings just like other

multidrug-resistant organisms (MDROs), such as methicillin-resistant Staphylococcus aureus (4 patients also had bacteremia caused by Enterobacter cloacae and S. haemolyticus).

Despite upgraded infection control measures, they say, the pandemic may provide "ideal conditions" for outbreaks of C. auris in hospital ICUs. They point out that both C. auris and SARS-CoV-2 have been found on hospital surfaces, including air conditioner ducts, windows, and hospital floors. C. auris can survive on a wide range of surfaces, both dry and moist, for up to 14 days. Disinfectants with sporicidal activity and hydrogen peroxide-based products are the most successful, so far, at reducing C. auris colony-forming units, and chlorine-based detergents, ultraviolet light, and hydrogen peroxide vapor have been effective in environmental decontamination.

Xerostomia could be one of the early symptoms of COVID-19 or due to medical comorbidities and drug reactions. Moreover, older patients may have difficulty in maintaining good oral health in the form of physical disabilities or psychological illnesses. It has been recently reported that Candida colonization was significantly associated with cognitive impairment, multimorbidity, and reduced oral hygiene capacity.

In conclusion, oral candidiasis has been consistently recorded in severely affected COVID-19 patients, especially the ones with predisposing comorbidities and antibiotics intake, either justified or unjustified. Older age and female gender seemed to be the most prominent demographic risk factors for this opportunistic infection, which tends to have late-onset and requires an immediate therapeutic intervention either systemically or topically to stop it from progression into lethal candidemia.[19]

iii. Aspergillosis

Caused by Aspergillus flavus, we should pay more attention to Aspergillus because invasive pulmonary aspergillosis (IPA) is difficult to diagnosis and can be associated with high morbidity and mortality. Like other fungal infections in covid patients, it also has been associated with uncontrolled diabetes, any previous respiratory pathology, immunosuppressive therapy, nosocomial infection sources and systemic immune alterations of Covid-19 infection.

Signs and symptoms:

a. Acute lethargy, feeling fatigue and/or unexplained exhaustion

Since the fungal infection spreads internally, it starts to weigh heavily on the internal organs that it affects. This could leave one feeling exhausted and lethargic.

b. Loss of appetite

The fungal infection can also interfere with the digestive system, which could lead to a loss of appetite.

c. Unusual and unexplained weight loss

Doctors recommend watching out for changes in metabolism. A sudden unexpected weight loss must be investigated.

d. Red and sunken eyes

While facial deformity is a symptom associated with Black Fungus (Mucormycosis), those suffering from Yellow Fungus can experience red and sunken eyes too.

e. Slow healing of wounds

In some cases, Yellow Fungus can cause a delay in healing of wounds, while in extreme cases, it may also lead to the leakage of pus. These conditions need medical attention immediately.

Diagnosis

Clinicians should consider the possibility of aspergillosis in patients with severe COVID-19 who have worsening respiratory function or sepsis, even if they do not have classical risk factors for aspergillosis. Testing for CAPA usually involves obtaining specimens from patient's lower respiratory tract, which are tested for Aspergillus galactomannan antigen and fungal culture.

Several radiographic findings, such as peripheral nodule, air crescent, reverse halo sign, nodular consolidation, ground-glass opacities, crazy paving pattern, pleural effusion, and pulmonary cysts were reported among patients with COVID-19-associated pulmonary aspergillosis.

Treatment

Voriconazole was the most commonly used antifungal agents, followed by caspofungin, Isavuconazole and liposomal amphotericin B.[19]

Coagulopathy in Covid-19 patients

In around 20% of infected patients, the initial immune response will not be sufficient to control viral replication because of aberrancies in the immune response or a high initial viral load, or both. These patients will develop more severe symptoms and proceed to stage 2, in which the uncontrolled viral replication will lead to apoptosis of pneumocytes and endothelial cells which in turn will activate platelets, induce coagulation factors, and lead to increased inflammation.

This cascade of events will result in further destruction of pneumocytes, pulmonary microangiopathy, and (inflammatory) microthrombi causing more severe symptoms and the need for additional oxygen supply; although, a relative balance between procoagulant and anticoagulant as well as proinflammatory and anti-inflammatory factors seems to be maintained. In approximately 5% of symptomatic patients, proinflammatory processes derail into a so-called cytokine storm. This cytokine storm will fuel proinflammatory and procoagulatory processes even further, which will result in systemic endotheliitis and capillary leakage, cellular dysfunction, organ dysfunction (including acute respiratory distress syndrome), and overt activation of the (systemic) coagulation cascade resulting in the need for critical organ support (stage 3). Although thrombogenicity of COVID-19 differs considerably from other severe infectious and non-infectious diseases, increased bleeding risk, especially in severely ill patients, remains a serious concern because bleeding complications are facilitated by thrombocytopenia, platelet dysfunction or coagulation factor deficiencies, or both, which are often present in critically ill patients with COVID-19.[20]

Decisions to care for these critically ill patients are complicated by the rapidly evolving data and anecdotes. It is therefore required of all of us caring for these patients to make as thoughtful and informed clinical decisions as we can. Based on a review of the very limited current peer- reviewed literature with low quality of evidence combined with discussions with international clinicians on the frontlines, the recommendations were:

i. All patients with COVID-19 should undergo coagulation studies at admission, in particular: D-dimer, prothrombin time, and platelet count.

ii. Because of the possibility of patients to develop coagulopathy later in their hospital course, routine serial measurements of coagulation studies should be undertaken in all COVID-19 patients. The ideal interval has not yet been defined.
iii. All patients with COVID-19 should be placed on prophylactic doses of anticoagulation, preferably with LMWH, unless there is a contraindication, such as acute kidney injury (AKI), wherein unfractionated heparin is preferred.
iv. Therapeutic anticoagulation should be strongly considered in patients at high-risk for coagulopathy (including CRRT and ECMO), demonstrating signs of microthrombi-induced organ dysfunction, or with documented or strongly suspected macro-thromboembolism. Determination of high-risk patients by laboratory measures of coagulopathy may include: platelet count, prothrombin time, fibrinogen, fibrinogen-degradation products, D-dimer, and TEG. Of note, some centers are therapeutically anticoagulating all patients on admission when no absolute contraindications exist.
v. Given the significant rate of AKI seen in COVID, intravenous contrast for imaging should be used with caution. Duplex ultrasonography, echocardiography, and clinical suspicion can play an increased role in these cases.
vi. Some early reports support extended-infusion tPA as a potential approach to refractory cases.
vii. Aspirin should be considered in cases with elevated troponin and cardiac dysfunction, particularly with elevated maximal amplitude on TEG.[20]

New-Onset Diabetes in Covid-19

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) exhibits increased mortality and morbidity in elderly individuals, especially in those with comorbidities, such as diabetes mellitus (DM). Previously, DM was identified as an independent factor predisposing to poor outcomes in patients infected by other coronaviruses, such as severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) and Middle East respiratory syndrome coronavirus (MERS-CoV). Moreover, during the SARS-CoV-1 outbreak, acute DM was commonly observed in individuals with no history of DM or glucocorticoid use and was an independent predictor of mortality.

Interestingly, DM may also be associated with severe coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2. Of note, "new-onset" hyperglycemia and acute metabolic decompensation of pre-existing DM are now emerging as a complication of COVID-19, especially among hospitalized patients. Impressively, this "new-onset" hyperglycemia is not associated with any other risk factors, notably obesity, prediabetes, DM, or corticosteroid administration. These findings point to a bidirectional relationship between DM and COVID-19.[21]

Type 1 DM is a genetic autoimmune condition where β-cells are destroyed by the auto-reactive CD4+ and CD8+ T cells. New onset diabetes in corona patients is of type 1.

First, some patient cases have illustrated that COVID-19 may accelerate diabetic ketoacidosis (DKA) in subjects with new-onset or pre-existing DM. Early recognition of DKA symptoms is required to improve the prognosis of COVID-19-related DKA.

Moreover, it is known that SARS-CoV-2 may enter the pancreatic beta cells via the expression of angiotensin-converting enzyme 2 (ACE2) receptor. It would be possible that the virus impairs pancreatic insulin secretion, thereby either aggravating DM or triggering new-onset DM. A further underlying mechanism appears to be insulin resistance due to the high levels of interleukin-6 (IL-6) and tumour necrosis factor alpha (TNFα) in subjects with severe COVID-19.

Vice versa, this new-onset hyperglycemia is linked to important perturbations. The latter include glycation of ACE2 receptors, excess cytokine release, and a pro-thrombotic state via increased antithrombin III production, ultimately leading to a more sinister prognosis. Indeed, a strong association of plasma glucose at admission with intubation and death has been demonstrated in DM.

In this context, there is accumulating evidence that hyperglycemia at admission, both in DM subjects and in those with secondary hyperglycemia, indicates a poor prognosis. Importantly, newly diagnosed DM is linked to increased mortality, as compared with known DM and normal glucose levels in patients with COVID-19. In the light of these findings,

several leading diabetologists have established a global registry of patients with COVID-19-related new DM to further investigate the intricacies and implications of these associations.

In conclusion, not only is DM associated with worse prognosis in COVID-19 but, vice versa, the latter may lead to new-onset DM, as well. Some mechanisms mediating this new hyperglycemia have been implicated. From a practical viewpoint, new hyperglycemia is linked to unfavorable prognosis, perhaps even more than in pre-existing DM. Hence, we need more knowledge, but we also need to deal with the emerging clinical implications, mastering the "fearful symmetry" of these new conditions.[21]

Long Covid

As the COVID-19 pandemic continues, the need to understand and respond to long COVID is increasingly pressing. Symptoms such as persistent fatigue, breathlessness, brain fog, and depression could debilitate many millions of people globally. Yet very little is known about the condition. The term "long COVID" is commonly used to describe signs and symptoms that continue or develop after acute COVID-19. A NICE guideline, for example, includes both ongoing symptomatic COVID-19 (from 4 to 12 weeks) and post-COVID-19 syndrome (≥12 weeks), but there is no agreed upon definition. How distinct is long COVID from other post-viral syndromes? No clear biochemical or radiological features exist to aid diagnosis, and there are potentially several phenotypes with different presentations, prognosis, and outcomes. With no proven treatments or even rehabilitation guidance, long COVID affects people's ability to resume normal life and their capacity to work. The effect on society, from the increased health-care burden and economic and productivity losses, is substantial. Long COVID is a modern medical challenge of the first order.

Most evidence about long COVID has been limited and based on small cohorts with short follow-up. Fatigue or muscle weakness was the most frequently reported symptom at both 6 months and 12 months, while almost half of patients reported having at least one symptom, such as sleep difficulties, palpitations, joint pain, or chest pain, at 12 months. The study shows that for many patients, full recovery from COVID-19 will take more than 1 year, and raises important issues for health services and research.[22]

Multisystem Inflammatory Syndrome (MIS)

Multisystem inflammatory syndrome (MIS) can affect children (MIS-C) and adults (MIS-A). MIS is a rare but serious condition associated with COVID-19 in which different body parts become inflamed, including the heart, lungs, kidneys, brain, skin, eyes, or gastrointestinal organs. COVID-19-associated multisystem inflammatory syndrome in children and adolescents is referred to interchangeably as paediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2 (PIMS-TS) or multisystem inflammatory syndrome in children (MIS-C) associated with COVID-19, and herein is referred to as MIS-C. MIS-C can lead to shock and multiple organ failure requiring intensive care. Children with MIS-C are very ill with a fever for 24 hours or more, have inflammation in their bodies and problems with many organs, such as the intestines, heart, brain, lungs, skin, and kidneys.

The symptoms are: Abdominal (gut) pain, bloodshot eyes, chest tightness/pain, diarrhea, fatigue, headache, low blood pressure, neck pain, rash, vomiting and fever. Diagnosis is based on elevated markers of inflammation such as ESR, C-reactive protein, or procalcitonin. No other obvious microbial cause of inflammation, including bacterial sepsis, staphylococcal or streptococcal shock syndromes.[23]

4. DIAGNOSTIC TESTS

Diagnostic tests are used to detect the presence or absence of disease in an individual. As it is a pandemic situation and number of cases are increasing rapidly, to curb the spread of the disease, detection and isolation of positive cases is of utmost importance. Keeping this in mind the general physicians who are attending these cases regularly in first instance, should know properly which test should be advised for the timely diagnosis.[24]

Diagnosis of COVID-19 requires detection of SARS-CoV-2 RNA or antigen in respiratory specimens. Detection of SARS-CoV-2 viral RNA is better in nasopharynx samples compared with throat samples. Lower respiratory samples may have better viral yield than upper respiratory samples.[24]

Sample requirement for covid-19 testing

Appropriate sample collection is the most important step in the laboratory diagnosis of any infectious disease. Improper specimen collection may contribute to false negative test results. The guideline mandates clinical sample collection by trained laboratory personnel/health-care workers in the presence of a clinician. Generally, samples for COVID-19 diagnosis are collected from two major sources: *upper respiratory tract and lower respiratory tract*. Upper respiratory tract specimens are collected by nasopharyngeal (NP) swab or the oropharyngeal (OP) swab, whereas the bronchoalveolar lavage, tracheal aspirate, or sputum will be collected from the lower respiratory tract. ICMR has created a comprehensive Specimen Referral Form for COVID-19 for use by all specimen collection centers and testing labs.

Nasopharyngeal Swabs

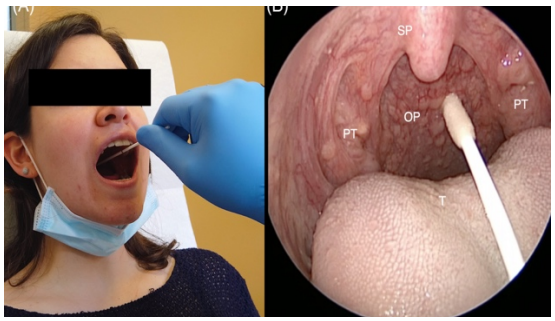
The NP specimen is a vital and sensitive sample to test the SARS-CoV-2 virus. As suggested by the Centers for Disease Control and Prevention (CDC), it is highly recommended to collect only the NP swab, although OP (oropharyngeal) swabs remain an acceptable specimen type. In the case that both NP and OP swabs are collected, they should be combined in a single tube to maximize sample load and test sensitivity. Synthetic fiber swabs with plastic shafts are recommended for NP and OP samples collection. Calcium alginate or wooden-shaft

swabs are not recommended because they might inactivate the virus and could provide a negative result. NP and OP samples should be placed immediately into an appropriate sterile medium or saline for proper transportation.

Courtesy: Francisco M Marty; How to Obtain a Nasopharyngeal Swab Specimen

Oropharyngeal Swabs

The OP swab is another important specimen recommended by the WHO and CDC to detect SARS-CoV-2 infection. The OP swab is collected from the posterior pharynx region, avoiding contact with the tongue.



Courtesy:Wiley online library

Bronchoalveolar Lavage and Tracheal Aspirate

Collection of the bronchoalveolar lavage (BAL) and tracheal aspirate is recommended only in severely ill and hospitalized patients.

Sputum

Sputum is collected from patients who have severe coughing symptoms. Sputum is collected by asking patients to expectorate deep-cough sputum directly straight into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container.

Other Samples

Blood and stool samples are also used in diagnosis of infection since SARS-CoV-2 is known to present in blood and stool. A small study reported the presence of SARS-CoV-2 in anal or oral swabs of patients in the Hubei Province. However, the utility of these samples remains unclear, because the data on viral shedding post-infection is still preliminary.[25]

Diagnostic tests recommended by ICMR

A. Real time Reverse transcriptase PCR for SARS-CoV-2:

This is a real-time reverse transcriptase polymerase chain reaction (rRT -PCR) test which can be run either in a singleplex format (individual targets in individual wells) or multiplexed formats (multiple targets in a single well). Amplification set up with a human RNase P (RNP) in a clinical sample as a human specimen control which is used to ascertain the quality of sample collection. RNA isolated from nasopharyngeal and oropharyngeal swabs, is reversely transcribed to form a Complementary DNA (cDNA) strand and then it is amplified multiple times using thermocycler machine which provides stringent conditions for the amplification reactions to happen. The fluorescence signals emitted from the Taqman probes is captured by the CCD camera and amplification plot is generated in the exponential phase.

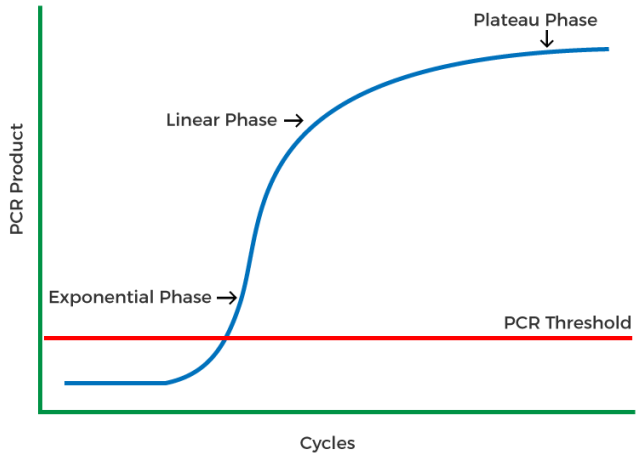
Conventional PCR is one of the most frequently used molecular technique for diagnosis of infectious disease. However, post amplification processing such agarose gel electrophoresis and less sensitivity of conventional PCR makes it an unsuitable approach for diagnosis of SARS-CoV2 as Covid-19 demands prompt diagnosis for better management and isolation of the patients. Real Time RT -PCR is a specialized version of PCR which can directly amplify the viral RNA from the clinical samples and obviates the need of post amplification end point analysis and the amplification which can be monitored in real time.[26]

Principle of Real time PCR:

Real-time PCR uses the technique of analysing data through the PCR where it includes the combination of single step amplification and detection through fluorescence capture

technique. It utilizes a different fluorescent dye that directly correlates genomic product concentration which is amplified to fluorescence intensity. Here, the reactions are characterized by the time point, where the target amplification is detected first in the exponential phase. This value is usually known to as cycle threshold (Ct), which implies the time at which detectable fluorescence intensity is higher than the background fluorescence. To paraphrase, the higher the quantity of genetic material existing in the clinical sample, the earlier significant increase in fluorescent signal will generate, yielding a lower Ct. The Real Time-PCR run for molecular diagnosis is carried out along with known positive controls, no amplification control and no template control.

The RT-PCR amplification curve has four phases, such as a) baseline b) exponential c) linear and d) plateau. The baseline phase is the one where all the amplification plots are below the detection level. The exponential phase is described as the earliest detectible fluorescent signal where the amplification of genetic material is taking place in the exponential phase which is dependent on the concentration of the template in the sample. This phase is followed by the linear amplification plot where the amplification begins to taper off where curve resembles as a straight line and the amplification plot gradually declines when it reaches the plateau phase.[26]



Courtesy: Medicinal Genomics

Methodology of Real time PCR:

Sample collection*:* The most commonly used samples for real time PCR are both nasopharyngeal swab and oropharyngeal swab. Under special conditions like when patient is in intubation, endotracheal secretion or Tracheal aspirate, BAL aspirate can also be collected. Many a times in particularly in postmortem cases nasal swabs can be tested. The samples are

collected in Viral Transport Medium (VTM) and are transported to the laboratory maintaining the proper cold chain. As SARS-COV 2 being a RNA virus, it needs cold environment to be stable so that it can be detected in Real time PCR.

Decontamination: It is the first step for processing of clinical samples in laboratory. As the sample is received in the lab, it is opened in a biosafety cabinet with proper PPE and then lysis buffer is added to inactivate the viral proteins and render the sample non-infectious. The average Time taken is 10-15 minutes per sample and it is performed in batches.

RNA Extraction: The Samples post proper decontamination are processed for RNA extraction. RNA extraction is done by commercial kits either manually or in an automated platform. For manual extraction time taken varies according to number of samples. On an average it takes around 1-2 hours for extraction of 96 samples. However, in case of an Automated extractor system it varies according to load capacity of the machines which ranges of 24, 64 and 96 samples at a single time and taking a time from one hour to 2 hours to complete the entire extraction.

Issue related to Interpretation of Ct value and Viral load:

It has been observed that Ct value is inversely proportion to the viral load theoretically. High viral load suggests increase infectiveness along with severity of the disease. However, this is a robust finding based on assumptions and limitations.

To clear the confusions related to Ct values as a guide for patient management, ICMR has released an advisory on the correlation of Ct values of real time RT-PCR test with COVID-19 disease severity. It has been observed that the Ct value varies according to the various kits used, the type of sample collected and sample collection procedure, transport procedure of the specimen etc., Moreover, an asymptomatic/mild symptomatic case might have same Ct value like that of a severe symptomatic case of COVID-19. This finding points to the conclusion that there is no direct correlation between the disease severity and the Ct values. Rather the disease severity and patient outcome depends on various factors like immune status of the patient, presence of co morbid conditions etc. One thing worth noting here is that the RT PCR that are being performed presently are qualitative in nature and doesn't measure or quantify the viral load.

Courtesy: thenewsminute.com

Advantages of RT-PCR for SARS-CoV-2:

i. High precision with increased sensitivity and specificity.
ii. It's a robust technique which is well acquainted by many medical staffs.
iii. Lots of samples can be processed together in 96 well microtiter plates.
iv. Due to presence of Positive and negative controls the results are validated in each PCR run minimizing the chances of false positive and false negative results
v. There is presence of human gene in most of the RT PCR kits as an extraction control which helps to determine whether the sample collected, and RNA extracted out of it is adequate.
vi. Different target genes of SARS-CoV-2 have been evaluated and validated in different kits. So, there is an option for choosing the right kit as per the need.

Disadvantages of RT-PCR for SARS-CoV-2:

i. Lots of technical expertise is required to perform and interpret the test.
ii. Reagents are to be transported and stored in controlled environment i.e., -20 degree Celsius.
iii. At least Biosafety level 2 (BSL) type of laboratory is required to perform this test, so its not for basic laboratories. Laboratories need to be designed with proper workflow. If proper biosafety practices are not performed there shall be risk of

occupational health hazards along with contamination of the samples which may interfere with the results giving rise to false positivity.

iv. The sensitivity and specificity vary based on the kits. So, results might vary and depends on subjective error due to human interference.

v. Time consuming as it requires multiple steps.

vi. Quality control and calibration of instruments are important to get accurate & validated results.

vii. High cost of consumables and various sophisticated equipment involved in Extraction and PCR process.

Apart from this the RT PCR for SARS-CoV-2 has following limitations:

Timeline of disease progression, type of sample and sample quality are other factors which contribute to diagnostic uncertainty. As long incubation time of the disease varies and in initial days of illness there is a low viral load it may produce a false negative result. In many cases reported false negative cases, patients did not carry enough viral load to be detected positive at the time of sampling.

Moreover, a negative result from a respiratory sample can only tell whether the virus is cleared from the respiratory tract but it's difficult to interpret whether it has been cleared from other body fluids or not.A positive test result for COVID-19 indicates that RNA from SARS-CoV-2 was detected, and the patient is presumptively infected with the virus. While the individual is presumed to be contagious, a limitation of all tests used to detect nucleic acid in respiratory pathogens is their inability to differentiate between infective and non-infective viruses. A negative result does not exclude the possibility of COVID-19.[26]

B. Cartridge based Nucleic acid amplification technique (CBNAAT), for Detection of SARS-COV-2

CBNAAT (GeneXpert) is an in-vitro qualitative nested real-time polymerase chain reaction. This cartridge-based automated molecular diagnostic modality was initially endorsed by WHO for detection of Mycobacterium tuberculosis rifampicin resistance for both pulmonary and extra pulmonary Tuberculosis in children within two hours.

Principle: This automated system is also based on the basic principle of rRT-PCR to detect SARS-COV2, but it integrates the various steps like the sample preparation, viral RNA extraction, amplification as well as detection of target sequences in a single cartridge. It uses single-time disposable cartridges that contains the RT-PCR reagents like primers, probes and internal control and perform the RT-PCR in GeneXpert Instrument systems. This system comprises of an instrument that holds cartridges computer with specific software for running tests and interpretation of graphs.

Sample type: Upper respiratory specimens such as nasopharyngeal and oropharyngeal swabs are the preferred samples for CBNAAT just like RT PCR for SARS-COV-2

The components of the system are;

i. Primers specific for the COV genes for the RNA detection from SARS-COV-2 in upper respiratory samples.
ii. Sample Processing Control (SPC): The SPC depicts for adequate sample processing and for the presence of potential inhibitor (s) in the RT-PCR reaction. The SPC also indicates that the reaction conditions like temperature, amplification time and reagents (primers, probes etc) for RT-PCR are functional.
iii. The Probe Check Control: This verifies that the components to perform the reaction are present in the cartridge, checks PCR tube filling, reagent rehydration as well as confirms and monitors the dye stability and probe integrity.

Method of Performing CBNAAT for SARS-CoV-2*:*

The upper respiratory specimens are collected in a Viral transport media (VTM) containing either 3 mL of VTM or 3 mL of saline and transported to lab maintaining the proper cold chain.

In the laboratory, the specimen after receiving in the laboratory is mixed by rapidly inverting the collection tube 5-6 times and then transferred to the sample chamber of the Xpert Xpress SARS-CoV-2 cartridge.

The cartridge containing the sample is loaded into the GeneXpert Instrument systems that performs the entire rRT-PCR.

Target Genes:

i. E (Envelope) gene: Screening gene for detection of SARS-COV2
ii. N2 (Nucleocapsid gene): Confirmatory gene for the SARS-COV2 virus

(Both these genes are detected simultaneously in single run only)

Result interpretation for SARS-COV-2:

Positive for SARS-COV2: If the Ct values for both N2 and E or only the N gene are within the valid range irrespective of the SPC that can be positive or negative, the sample is considered positive. The negative SPCs can be ignored as the target amplification has occurred.

Presumptive Positive for SARS-COV2: If the SARS-COV-2 signals for only the E nucleic acid target irrespective of the SPC that can be positive or negative. The negative SPCs can be ignored as the target amplification has occurred.

In this case sample can be retested and if the same result is coming, the resample of the patient can be asked after 5-7 days of initial test.

Negative result for SARS-COV2: SARS-COV-2 target N2 and E gene are not detected.

Advantages of CBNAAT for SARS-CoV-2:

i. Cross-contamination between samples is minimized as the cartridges are self-contained.
ii. This system has a quick turnaround time (approx. 1 hr 45 minutes) that includes the nucleic acid extraction time.
iii. The screening and confirmatory genes are done in single run.
iv. The confirmatory gene N, nucleocapsid gene is specific for SARS-COV-2

v. This platform has widespread availability even at district and primary health centre level as it has been widely used for diagnosis of Tuberculosis and other infectious diseases.

Limitations of CBNAAT for SARS-CoV-2:

i. The proper temperature control as well as annual calibration of instrument is must.
ii. Uninterrupted power supply is required (with additional batteries or a generator can be attached).
iii. Performance evaluation and validation of CBNAAT results for SARS-CoV-2 was mainly done on nasopharyngeal swabs and other nasal specimens such as nasal wash/aspirate. Validation yet to be performed on other upper respiratory samples such as oropharyngeal swab, nasal swab
iv. Factors such as improper and inadequate sample collection and transportation may affect the quality of the results giving rise to false negative results in such samples.
v. Ongoing mutations within the target sequence of SARS- CoV-2 genes such as S and N2, can alter the configuration of binding sites for primer and/or probe leading to the failure of the amplification process.[26]

C. Truenat testing for SARS-COV-2 detection:

Truenat technology for detection of SARS-COV-2 is a Make in India technology which was already in use in various RTNCP centres across India in the diagnosis of Pulmonary tuberculosis. Hence, in the present COVID-19 pandemic, the company has introduced a new platform and chips in the existing Truenat machines for detection of SARS-COV-2, which is an important addition in the ongoing quest for robust diagnostic methods for COVID19.

Principle:

Truenat technology for detection of SARS-COV-2 is again a real time reverse transcriptase PCR which uses a chip-based platform. This diagnostic platform uses two different chips for quantitative detection of beta coronavirus (sarbeco virus) and SARS-CoV-2 RNA respectively.

Target Genes:

i. E (Envelope) gene: Screening gene for detection of beta coronavirus (Truenat beta Cov chip)
ii. Rdrp (RNA dependent RNA polymerase) gene: Confirmatory gene for final detection of SARS-CoV-2 (Truenat SARS-CoV-2 chip)

Sensitivity & Specificity of the kit has been mentioned as 100% in company product brochure and they have also claimed that there is no cross reactivity to any other respiratory pathogen. NPS/OPS samples are received in a specialized vial containing lysis buffer made for Truenat (Point to note: sample sent for Truenat in this special vial cannot be tested for conventional RT PCR or CBNAAT). The lysis buffer inactivates the virus (if it is present in the sample) making it non-infectious. Therefore, biosafety requirements are minimal while performing Truenat testing.

Time taken for completion of the test:

RNA extraction time :18 minutes.

Truenat beta Cov (E gene detection) and Truenat SARS-CoV-2 testing time: 42 min.

So, the confirmed negative result can be obtained in around one hour (extraction time plus detection of E gene) and for screening test positive result another 42 min is required to get the confirmed positive/negative results for SARS-CoV-2.

Result Interpretation:

The progress of the test can be visualized on the screen of the analyzer by observing the two amplification curves
POSITIVE Result: It is indicated by the rise of Target graphs (E/RdRp) as well as the internal positive control (IPC) graphs in an exponential fashion and fluorescence crossing the threshold value. Rise of IPC curve is essential for the validity of the test result.

NEGATIVE result: There is no rise of the target graphs and they remain horizontal throughout the amplification process. Only the IPC graph shows an exponential rise indicating that the test run was valid.

RESULT INVALID: There is no rise of IPC curve and it remains horizontal at the end of the test. (Invalid samples should be repeated with fresh specimen from the sample preparation stage).

The positive results are also accompanied by icons like "HIGH", "MEDIUM", "LOW" or "VERY LOW" corresponding to the viral load of each sample as claimed by the kit instruction.[26]

Guidelines issued from ICMR regarding performing and reporting of TRUENAT tests for SARS-CoV-2

According to the ICMR advisory, all samples of suspected SARS-CoV-2 infection should be tested for E gene assay (Truenat Beta Cov Chip) first while using Truenat platform. The samples which are tested negative for E gene are considered as True Negative or Confirmed negative.

All the samples that are tested positive by E gene assay (Truenat Beta Cov chip) are further tested for of Rdrp gene (Truenat SARS-COV-2 chip) which is a confirmatory gene for SARS-CoV-2. The samples that are tested positive by this final assay for Rdrp are considered as True positive or Confirmed positive and there is no need of further RT PCR testing for these positive samples. Recently, Multiplex Truenat Assay has also been developed and it has been approved by ICMR. This assay uses E gene as screening and ORf1ab as confirmatory gene for COVID 19 detection in a multiplex format.

Advantages of Truenat testing:

i. Minimal Biosafety requirements as the sample is in a specialized medium where due to lysis buffer the virus become inactivated/noninfectious. That is why no special infrastructure is required and a basic healthcare facility can perform the tests
ii. Less technical expertise needed compared to conventional PCR.

iii. The results are easy to interpret as the results are available on the screen as detected/not detected /invalid.
iv. Less time consuming then PCR for setting up the test and time taken for the complete process of detection is also less compared to PCR.
v. A single sample can be put without wasting reagents. Helpful for emergency cases.
vi. Machine is small (tabletop) and doesn't require much space in the laboratory.

Disadvantages of Truenat testing:

i. Only one sample can be processed by one Truenat machine at a time. So not an ideal instrument for a high throughput laboratory.
ii. Specialized vial is required to transport the sample.
iii. The sample in Truenat VTM can't be used for conventional RT PCR or CBNAAT.
iv. Doesn't show you any graph at the end of the test. So there is no chance of analysis of the characteristics of the graph and the corresponding result.
v. In rare circumstances, mutations occurring within the highly conserved areas of the target genome where the Truenat assay primers and probes bind may result in false negative results.[26]

Category of patients for RT PCR testing of SARS-COV-2:

i. Influenza like illness cases with contact history with positive patients.
ii. Low risk symptomatic contacts of positive persons.
iii. Any adult patients with influenza like illness suspected of SARS-CoV-2 without co-morbidities with mild symptoms not requiring immediate intervention.
iv. Healthcare workers who require testing with high to medium risk of exposure.
v. Symptomatic healthcare workers and other frontline workers.
vi. Patients coming from containment zone requiring admission in the hospital.

Category of patients for CBNAAT & Truenat testing of SARS-COV-2:

i. All Severe acute respiratory illness (SARI) patients requiring immediate intervention.
ii. Dead bodies.
iii. Pediatric patients with SARI waiting to be transferred to intensive care.
iv. Before emergency surgical procedures wherever testing of SARS Cov is indicated.
v. Before delivery of pregnant lady coming from containment zone.

Key points to be noted while categorizing the patients for SARS-COV-2 testing:

It is important to remember that the above patient selection criteria for each test is arbitrary and might vary from institutions to institution and depends on the sample load of a particular testing facility. Moreover, as the number of samples that can be tested at a time in CBNAAT and Truenat varies depending on the number and type of the machines available, every institution should note this point before deciding the turnaround time (TAT) and selection of patient categories for each testing modalities.

Note: Considered the gold standard for Covid tests, the RT-PCR can take a day to declare results and involves sample collections and testing. Although it runs the advantage of testing almost 92 samples at a time, CBNAAT is much quicker and produces results within 45 minutes. The advantage of CBNAAT or TrueNAT(Indian variant) is that the machines are available across the country even in the remotest places. CBNAAT was also used in the National Tuberculosis Control program. Based on the cartridges system, the Covid cartridge was launched recently and is being used rampantly across the world. Since the technology is imported, the cost of a cartridge is around Rs 3000 and the testing costs go above Rs. 4000 per sample. Also, another limitation is that it can run only four tests at one time which limits the number of samples tested.[26]

D. Antigen testing

These tests are designed to detect a specific protein in the virus that elicits the body's immune response. In the case of Covid-19, it is the 'spike protein' present on the surface of the coronavirus that facilitates its entry into the human cell.

For this test, professionals collect a nasal swab, which is then immersed in a solution that deactivates the virus. A few drops of this solution are then put on a test strip. This has to be done within an hour of the immersion of the swab in the solution.

The test strips contain artificial antibodies designed to bind to coronavirus proteins. If a person is infected with coronavirus, the test lines will appear on the paper strips within 15 minutes.

Since antigen testing does not involve any amplification process, swab samples may lack enough antigen material to be detectable. This may result in false negative tests.

For this reason, if a person tests negative through antigen testing, they still need to get an RT-PCR test done for confirmation. If a person tests positive, however, a confirmation RT-PCR is not required.

The advantage of using this test is that it reduces the burden of relying on just RT-PCR tests to identify Covid-19 patients.

Antigen testing is useful because even if it's less sensitive, it is rapid and the results that are positive will be positive. So, patients who test positive can get into isolation faster.

The sensitivity of antigen tests reportedly varies from 34 to 80%, which means there could be nearly 50% of false negative results, depending on the group of patients tested. In September 2020, ICMR issued advisory recommending the use of a rapid antigen test (RAT) as an initial test for surveillance in containment zones and for screening at points of entry. Negative RAT result should always be confirmed by RT-PCR/TruNat/CBNAAT. Whereas in noncontainment zones and in hospital settings, RT-PCR/TruNat/CBNAAT is recommended for initial screening and followed by RAT.[27]

Courtesy: economictimes.indiatimes.com

E. Antibody tests

Antibody tests, also known as serological tests, detect whether a person has antibodies to the virus. Antibodies are naturally produced by the body's immune system to fight off infections. Antibody tests cannot be used to diagnose Covid-19 but can reveal whether a person was recently exposed to the virus.

Persons with antibodies in their bloodstream are likely to have immunity to the disease, although scientists are still not sure how long Covid-19 antibodies offer protection from the infection.

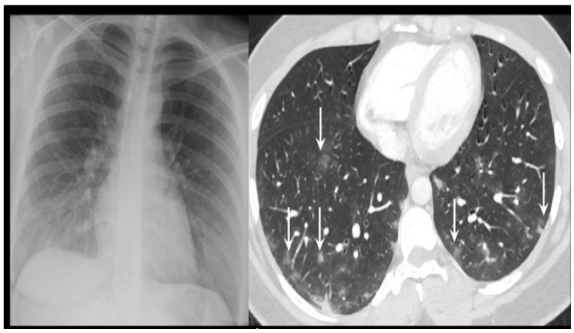
For the antibody test, trained professionals collect a few drops of blood. The sample is placed on a cassette or cartridge that contains the SARS-CoV-2 proteins. If the blood samples contain antibodies, they will immediately bind to the viral proteins. The positive result is indicated in the form of lines, like a home pregnancy test.

Antibody tests can be of different types. One of the tests, known as Enzyme-Linked Immunosorbent Assay (ELISA), which is designed specifically for screening large numbers of specimens at a time, is suitable for detecting the spread of the disease in a large population within a short span of time.

Antibody tests can be useful to carry out surveys to check whether a population has been exposed to the virus. These are currently only being used for research and surveillance purposes.[28]

F. CT Scans

High-resolution CT (HRCT) is extremely sensitive and the method of choice for diagnosing COVID-19 pneumonia, even in initial stages of the illness. The most commonly seen features are multifocal bilateral 'ground-glass' areas associated with consolidation and a patchy peripheral distribution, with greater involvement of the lower lobes. A 'reversed halo sign' is also seen in some patients, which is identified as a focal area of patchy opacities surrounded by a peripheral ring with consolidation. Other findings include pleural effusion, cavitation, calcification, and lymphadenopathy.[29]



CT sensitivity is considered to be above 90%. In other words: in at least 90 of 100 infected people the infection will be detected. That sounds good but can be a problem when the disease is not widespread. 'With a prevalence of less than 10%, CT is not suited for screening or primary diagnosis, since potentially there will be too many false positives,' she explained. Other viral infections can yield very similar CT findings, which is important above all in winter months. A cardiac oedema, or an acute form of interstitial fibrosis (NSIP), for example, are typical non-infectious mimics of Covid-19 – some patients present with fever and cough, and, on the images, the lung tissue changes related to these conditions look very much like the changes caused by Covid-19.[30]

As the variants are on rise, apart from relying only on RT-PCR, clinical symptoms and CT scan reports should be used as the guiding factor for treatment.

G. RT-LAMP (Reverse Transcriptase Loop Mediated Isothermal Amplification)

This is a one-step nucleic acid amplification method to multiply specific sequences of RNA of the coronavirus. Here, the RNA is first made into cDNA by the usual reveres transcription. Then, the DNA is amplified by the LAMP technique.

It has many advantages over RT-PCR technique. The RT-PCR technique needs different temperatures in one cycle. The temperature of the solution has to be changed from 92 degrees to 56 degree Celsius and again to 72 degree C every 2 minutes, and this cycle has to be repeated. Thus, the PCR tests needs expensive thermal cycler as well as the real time PCR machines. On the other hand, the new RT-LAMP technology is done at 65degree C, whereas the DNA amplification is done at constant temperature(isothermal), so that expensive thermal cycler is not required.

Moreover, the quantity of DNA amplified in the LAMP technology is hundred thousand times more than that is taking place in PCR. The assay is so fast that the results are obtained within 30minutes, and positive samples are amplified as early as 10minutes.

The LAMP technology has been recently validated by ICMR with sensitivity 98.7% and specificity 100%. Thus it is superior to PCR technology based COVID -19 kits where specificity is around 95% only.[31]

5. TREATMENT PROTOCOL

Initially, early in the pandemic, the understanding of COVID-19 and its therapeutic management was limited, creating an urgency to mitigate this new viral illness with experimental therapies and drug repurposing. Since then, due to the intense efforts of clinical researchers globally, significant progress has been made, which has led to a better understanding of not only COVID-19 and its management but also has resulted in the development of novel therapeutics and vaccine development at an unprecedented speed.[32]

Pharmacologic Therapies In The Management Of COVID-19

According to CDC, two main processes are thought to drive the pathogenesis of COVID-19. Early in the clinical course, the disease is primarily driven by replication of SARS-CoV-2. Later in the clinical course, the disease appears to be driven by a dysregulated immune/inflammatory response to SARS-CoV-2 that leads to tissue damage. Based on this understanding, it is anticipated that antiviral therapies would have the greatest effect early in the course of the disease, while immunosuppressive/anti-inflammatory therapies are likely to be more beneficial in the later stages of COVID-19.

No therapy has been proven to be beneficial in outpatients with mild to moderate COVID-19 who are not at high risk for disease progression. The COVID-19 Treatment Guidelines Panel (the Panel) recommends providing supportive care and symptomatic management to outpatients with COVID-19; steps should also be taken to reduce the risk of SARS-CoV-2 transmission to others.

Antiviral Therapies

vii. **Hydroxychloroquine and chloroquine** were proposed as antiviral treatments for COVID-19 initially during the pandemic. However, data from randomized control trials evaluating the use of hydroxychloroquine with or without azithromycin in hospitalized patients ***did not improve*** the clinical status or overall mortality compared to placebo. Data from randomized control trials of hydroxychloroquine used as postexposure prophylaxis ***did not prevent*** SARS-CoV-2 infection or symptomatic COVID-19 illness.

viii. **Ivermectin** is an FDA-approved anti-parasitic drug used worldwide in the treatment of COVID-19 based on an *in vitro* study that showed inhibition of SARS-CoV-2 replication. A single-center double-blind, randomized control trial involving 476 adult patients with mild COVID-19 illness was randomized to receive ivermectin 300 mcg/kg body weight for five days or placebo did not achieve significant improvement or resolution of symptoms. Ivermectin is ***currently not indicated*** for the treatment of COVID-19 in hospitalized and nonhospitalized patients.

ix. **Lopinavir/ritonavir** is an FDA-approved combo therapy for the treatment of HIV and was proposed as antiviral therapy against COVID-19 during the early onset of the pandemic. Data from a randomized control trial that reported no benefit was observed with lopinavir-ritonavir treatment compared to standard of care in patients hospitalized with severe COVID-19. Lopinavir/Ritonavir ***is currently not indicated*** for the treatment of COVID-19 in hospitalized and nonhospitalized patients.

x. **Remdesivir** is a broad-spectrum antiviral agent that previously demonstrated antiviral activity against SARS-CoV-2 *in vitro*. Based on results from three randomized, controlled clinical trials that showed that remdesivir was superior to placebo in shortening the time to recovery in adults who were hospitalized with mild-to-severe COVID-19, the U.S. Food and Drug Administration (FDA) approved remdesivir for clinical use in adults and pediatric patients (over age 12 years and weighing at least 40 kilograms or more) to treat hospitalized patients with COVID-19. However, results from the WHO SOLIDARITY Trial conducted at 405 hospitals spanning across 40 countries involving 11,330 inpatients with COVID-19 who were randomized to receive remdesivir (2750) or no drug (4088) found that remdesivir had little or no effect on overall mortality, initiation of mechanical ventilation, and length of hospital stay. There is no data available regarding the efficacy of remdesivir against the new SARS-CoV-2 variants; however, acquired resistance against mutant viruses is a potential concern and should be monitored.

Anti-SARS-CoV-2 Neutralizing Antibody Products

Individuals recovering from COVID-19 develop neutralizing antibodies against SARS-CoV-2, and the duration of how long this immunity lasts is unclear. Nevertheless, their role as

therapeutic agents in the management of COVID-19 is extensively being pursued in ongoing clinical trials.

i. **Convalescent Plasma** therapy was evaluated during the SARS, MERS, and Ebola epidemics; however, it lacked randomized control trials to back its actual efficacy. The FDA approved convalescent plasma therapy under a EUA for patients with severe life-threatening COVID-19. Although it appeared promising, data from multiple studies evaluating the use of convalescent plasma in life-threatening COVID-19 has generated mixed results. One retrospective study based on a U.S. national registry reported that among patients hospitalized with COVID-19, not on mechanical ventilation, there was a lower risk of death in patients who received a transfusion of convalescent plasma with higher anti-SARS-CoV-2 IgG antibody than patients who received a transfusion of convalescent plasma with low antibody levels. Data from three small randomized control trials showed ***no significant differences in clinical improvement*** or overall mortality in patients treated with convalescent plasma versus standard therapy. An in vitro analysis of convalescent plasma obtained from individuals previously infected with the ancestral SARS-CoV-2 strains demonstrated significantly reduced neutralization against SARS-CoV-2 variant B.1.351/ 501Y.V2. Another in vitro study reported B.1.351 variant exhibited markedly more resistance to neutralization by convalescent plasma obtained from individuals previously infected with the ancestral SARS-CoV-2 strains compared to the B.1.1.7 variant, which was not more resistant to neutralization.

ii. **REGN-COV2 (Casirivimab and Imdevimab):** REGN-COV2 is an antibody cocktail containing two noncompeting IgG1 antibodies (casirivimab and imdevimab) that target the RBD on the SARS-CoV-2 spike protein that has been shown to decrease the viral load *in vivo*, preventing virus-induced pathological sequelae when administered prophylactically or therapeutically in non-human primates. Results from an interim analysis of 275 patients from an ongoing double-blinded trial involving non hospitalized patients with COVID-19 who were randomized to receive placebo, 2.4 g of REGN-COV2 (casirivimab 1,200 mg and imdevimab 1,200 mg) or 8 g of REGN-COV2 COV2 (casirivimab 2,400 mg and imdevimab 2,400 mg) reported that the REGN-COV2 antibody cocktail reduced viral load compared to placebo. This interim analysis also established the safety profile of this cocktail antibody, similar to that of the placebo group. Preliminary data from a Phase 3 trial of REGN-COV (casirivimab/imdevimab) revealed a 70% reduction in hospitalization or death in nonhospitalized patients with COVID-19. In vitro data is available regarding the effect of

REGN-COV2 on the two new SARS-CoV-2 variants of concern (B.1.1.7; B.1.351 variants) that reveal retained activity.

iii. **Bamlanivimab and Etesevimab** are potent anti-spike neutralizing monoclonal antibodies. Bamlanivimab is a neutralizing monoclonal antibody derived from convalescent plasma obtained from a patient with COVID-19. Like REGN-COV2, it also targets the RBD of the spike protein of SARS-CoV-2 and has been shown to neutralize SARS-CoV-2 and reduce viral replication in non-human primates. *In vitro* experiments revealed that etesevimab binds to a different epitope than bamlanivimab and neutralizes resistant variants with mutations in the epitope bound by bamlanivimab. In Phase 2 of the BLAZE-1 trial, bamlanivimab/etesevimab was associated with a significant reduction in SARS-CoV-2 viral load compared to placebo. Data from the Phase 3 portion of BLAZE-1 is pending release, but preliminary information indicates that therapy reduced the risk of hospitalization and death by 87%. *In vitro* data is available regarding the effect of bamlanivimab/etesevimab on the new SARS-CoV-2 variants of concern (B.1.1.7; B.1.351) reveals retained activity.

iv. **Sotrovimab(VIR-7831)** is a potent anti-spike neutralizing monoclonal antibody that demonstrated *in vitro* activity against all the four VOCs Alpha (B.1.1.7), Beta (B.1.351), Gamma(P1), and Delta (B.1.617.2). Results from a preplanned interim analysis(not yet peer-reviewed) of the multicenter, double-blind placebo-controlled Phase 3, COMET-ICE trial by Gupta *et.al* that evaluated the clinical efficacy and safety of sotrovimab demonstrated that one dose of sotrovimab(500 mg) reduced the risk of hospitalization or death by 85% in high-risk non hospitalized patients with mild to moderate COVID-19 compared with placebo.

Note: REGN-COV2 (casirivimab and imdevimab),bamlanivimab/etesevimab, and sotrovimab were approved for clinical use by the FDA under three separate EUAs issued in November 2020, February 2021, and May 2021, respectively, that allowed the use of these drugs only in nonhospitalized patients (aged ≥12 years and weighing ≥40 kg) with laboratory-confirmed SARS-CoV-2 infection and mild to moderate COVID-19 who are at high risk for progressing to severe disease and/or hospitalization.

Immunomodulatory Agents

i. **Corticosteroids:** Severe COVID-19 is associated with inflammation-related lung injury driven by the release of cytokines characterized by an elevation in inflammatory markers. During the pandemic's early course, glucocorticoids' efficacy in patients with COVID-19 was not well described. The Randomized Evaluation of Covid-19 Therapy (RECOVERY) trial, which included hospitalized patients with clinically suspected or laboratory-confirmed SARS-CoV-2 who were randomly assigned to received dexamethasone (n=2104) or usual care (n=4321), showed that the use of dexamethasone resulted in lower 28-day mortality in patients who were on invasive mechanical ventilation or oxygen support but not in patients who were not receiving any respiratory support. Based on the results of this landmark trial, dexamethasone is currently considered the standard of care either alone or in combination with remdesivir based on the severity of illness in hospitalized patients who require supplemental oxygen or non-invasive or invasive mechanical ventilation.

ii. **Interferon-β-1a (IFN- β-1a):** Interferons are cytokines that are essential in mounting an immune response to a viral infection, and SARS-CoV-2 suppresses its release *in vitro*. However, previous experience with IFN- β-1a in acute respiratory distress syndrome (ARDS) has not benefited. Results from a small randomized, double-blind, placebo-controlled trial showed the use of inhaled IFN- β-1a had greater odds of clinical improvement and recovery compared to placebo. Another small randomized clinical trial showed that the clinical response using inhaled IFN- β-1a was not significantly different from the control group. The authors reported when used early, this agent resulted in a shorter length of hospitalization stay and decreased 28-day mortality rate. However, four patients who died in the treatment group before completing therapy were excluded, thus making the interpretation of these results difficult. Currently, there is no data available regarding the efficacy of interferon β-1a on the four SARS-CoV-2 VOCs Alpha (B.1.1.7), Beta (B.1.351), Gamma(P1), and Delta (B.1.617.2). Given the insufficient and small amount of data regarding this agent's use and the relative potential for toxicity, this therapy is not recommended to treat COVID-19 infection.

iii. **Interleukin (IL)-1 Antagonists:** Anakinra is an interleukin-1 receptor antagonist that is FDA approved to treat rheumatoid arthritis. Its off-label use in severe COVID-19 was assessed in a small case-control study trial based on the rationale that the severe COVID-19 is driven by cytokine production, including interleukin (I.L.)-1β. This trial revealed that of the 52 patients

who received anakinra and 44 patients who received standard of care, anakinra reduced the need for invasive mechanical ventilation and mortality in patients with severe COVID-19. There is no data available regarding the efficacy of interleukin-1 receptor antagonists on the three new SARS-CoV-2 variants (B.1.1.7; B.1.351, and P.1). Given the insufficient data regarding this treatment based on case series only, this is not currently recommended to treat COVID-19 infection.

iv. **Anti-IL-6 receptor Monoclonal Antibodies:** Interleukin-6 (IL-6) is a proinflammatory cytokine that is considered the key driver of the hyperinflammatory state associated with COVID-19. Targeting this cytokine with an IL-6 receptor inhibitor could slow down the process of inflammation based on case reports that showed favorable outcomes in patients with severe COVID-19. The FDA approved three different types of IL-6 receptor inhibitors for various rheumatological conditions (Tocilizumab, Sarilumab) and a rare disorder called Castleman's syndrome (Siltuximab).

v. **Tocilizumab** is an anti-interleukin-6 receptor alpha receptor monoclonal antibody that has been indicated for various rheumatological diseases. The data regarding the use of this agent is mixed. A randomized control trial involving 438 hospitalized patients with severe COVID-19 pneumonia, among which 294 were randomized to receive tocilizumab and 144 to placebo, showed that tocilizumab did not translate into a significant improvement in clinical status or lower the 28-day mortality compared to placebo. Results from another randomized, double-blind placebo-controlled trial involving patients with confirmed severe COVID-19 that involved 243 patients randomized to receive tocilizumab or placebo showed that the use of tocilizumab was not effective in preventing intubation or death rate. The REMAP-CAP and RECOVERY trials (not yet published), two large randomized controlled trials, showed a mortality benefit in patients exhibiting rapid respiratory decompensation.

vi. **Sarilumab and Siltuximab** are IL-6 receptor antagonists that may potentially have a similar effect on the hyperinflammatory state associated with COVID-19 as tocilizumab. Currently, there no known published clinical trials supporting the use of siltuximab in severe COVID-19. Conversely, a 60-day randomized, double-blind placebo control multinational phase 3 trial that evaluated the clinical efficacy, mortality, and safety of sarilumab in 431 patients did not show any significant improvement in clinical status or mortality rate.

Janus kinase (JAK) inhibitors

i. **Baricitinib** is an oral selective inhibitor of Janus kinase (JAK) 1 and JAK 2 currently indicated for moderate to severely active rheumatoid arthritis(RA) patients. Baricitinib was considered a potential treatment for COVID-19 based on its inhibitory effect on SARS-CoV-2 endocytosis *in vitro* and on the intracellular signaling pathway of cytokines that cause the late-onset hyperinflammatory state that results in severe illness. This dual inhibitory effect makes it a promising therapeutic drug against all stages of COVID-19. A multicenter observational, retrospective study of 113 hospitalized patients with COVID-19 pneumonia who received baricitinib combined with lopinavir/ritonavir (baricitinib arm, n=113) or hydroxychloroquine and lopinavir/ritonavir (control arm, n=78) reported significant improvement in clinical symptoms and 2-week mortality rate in the baricitinib arm compared with the control arm. Results from the ACTT-2 trial, a double-blind, randomized placebo-controlled trial evaluating baricitinib plus remdesivir in hospitalized adult patients with COVID-19, reported that the combination therapy of baricitinib plus remdesivir was superior to remdesivir therapy alone in not only reducing recovery time but also accelerating clinical improvement in hospitalized patients with COVID-19, particularly who were receiving high flow oxygen supplementation or noninvasive ventilation. Baricitinib, in combination with remdesivir, has been approved for clinical use in hospitalized patients with COVID-19 under a EUA issued by the FDA. The efficacy of baricitinib alone or in combination with remdesivir has not been evaluated in the SARS-CoV-2 variants, and there is limited data on the use of baricitinib with dexamethasone.

ii. **Ruxolitinib** is another oral selective inhibitor of JAK 1 and 2 that is indicated for myeloproliferative disorders, polycythemia vera, and steroid-resistant GVHD. Similar to baricitinib, it has been hypothesized to have an inhibitory effect on cytokines' intracellular signaling pathway, making it a potential treatment against COVID-19. Results from a small prospective multicenter randomized controlled phase 2 trial evaluating the efficacy and safety of ruxolitinib reported no statistical difference than the standard of care. However, most of the patients demonstrated significant chest C.T. improvement and faster recovery from lymphopenia. A large randomized, double-blind, placebo-controlled multicenter trial is ongoing to assess ruxolitinib's efficacy and safety in patients with severe COVID-19.

iii. **Tofacitinib** is another oral selective inhibitor of JAK 1 and JAK3 that is indicated for moderate to severe RA, psoriatic arthritis, and moderate to severe ulcerative colitis. Given its inhibitory effect on the inflammatory cascade, it was hypothesized that its use could ameliorate the viral inflammation-mediated lung injury in patients with severe COVID-19. Results from a small randomized controlled trial that evaluated the efficacy involving 289 patients who were randomized to receive tofacitinib or placebo showed that tofacitinib led to a lower risk of respiratory failure or death.

iv. **Bruton's tyrosine kinase inhibitors** such as **acalabrutinib, ibrutinib, rilzabrutinib** are tyrosine kinase inhibitors that regulate macrophage signaling and activation currently FDA approved for some hematologic malignancies. It is proposed that macrophage activation occurs during the hyperinflammatory immune response seen in severe COVID-19. Results from a small off-label study of 19 hospitalized patients with severe COVID-19 who received acalabrutinib highlighted the potential clinical benefit of BTK inhibition. Clinical trials are in progress to validate the actual efficacy of these drugs in severe COVID-19 illness.[32]

Oxygenation And Ventilation Management In COVID-19

Conventional Oxygen Therapy

COVID-19 patients with associated respiratory insufficiency should be monitored closely with continuous pulse oximetry. Supplemental oxygen supplementation via nasal cannula or Venturi mask must be administered to maintain oxygen saturation (SpO2) between 92 to 96% (< 88-90% if COPD). If there is improvement in clinical and oxygen saturation, supplemental oxygen should be continued with periodic reassessment. If there is no clinical improvement or worsening of symptoms and/or oxygen saturation, non-invasive treatments such as High-Flow Nasal Cannula (HFNC) or Non-invasive Positive Pressure Ventilation(NIPPV) are recommended**.**

Management of Acute Hypoxemic Respiratory Failure in COVID-19

Acute hypoxemic respiratory failure is the most common complication in adult patients with COVID-19, and conventional oxygen therapy is not helpful to address the oxygen demand in these patients. These patients should be managed with enhanced respiratory support modalities such as high-flow nasal cannula (HFNC), non-invasive positive pressure ventilation

(NIPPV), endotracheal intubation, and invasive mechanical ventilation (IMV) or extracorporeal membrane oxygenation (ECMO**).**

High-Flow Nasal Cannula (HFNC) and Non-invasive Positive Pressure Ventilation (NIPPV)

HFNC and NIPPV are non-invasive enhanced respiratory support modalities available in managing COVID-19-associated acute hypoxemic respiratory failure and are instrumental in avoiding invasive mechanical ventilation in carefully selected patients. A meta-analysis study evaluating the effectiveness of HFNC compared to conventional oxygen therapy and NIPPV before mechanical ventilation reported that HFNC, when used before mechanical ventilation, could improve the prognosis of patients compared to conventional oxygen therapy and NIPPV. The use of HFNC or NIPPV is associated with decreased dispersion of exhaled air especially when used with a good interface fitting, thus creating a low risk of nosocomial transmission of the infection. However, these treatment modalities are associated with a greater risk of aerosolization and should be used in negative pressure rooms.

Non-invasive Positive-pressure Ventilation (NIPPV)

i. NIPPV (bilevel positive airway pressure [BiPAP]/continuous positive airway pressure [CPAP]) is instrumental in the management of COVID-19-associated acute hypoxemic respiratory failure and may help avoid invasive mechanical ventilation in carefully selected patients.

ii. NIPPV should be restricted to hospitalized patients with COVID-19 who develop respiratory insufficiency due to COPD, cardiogenic pulmonary edema, or have underlying obstructive sleep apnea (OSA) rather than ARDS.

iii. A helmet is preferred for minimizing the risk of aerosolization. In NIPPV with face masks (full-face or oronasal), the use of masks integrated with an expiratory valve fitted with an antimicrobial filter is recommended.

iv. Results from the HENIVOT trial, an Italian open-label multicenter randomized clinical trial, reported that there was no significant difference in the number of days free of respiratory support with the utilization of helmet noninvasive ventilation treatment compared to high flow nasal oxygen in COVID-19 patients hospitalized with moderate to severe degree of hypoxemia.[33]

Endotracheal Intubation and Lung Protective Invasive Mechanical Ventilation

i. Impending respiratory failure should be recognized as early as possible, and a skilled operator must promptly perform endotracheal intubation to maximize first-pass success.

ii. Clinicians and other healthcare staff must wear appropriate PPE that includes gowns, gloves, N95 masks, and eye protection when performing endotracheal intubation and manual ventilation before intubation, physical proning of the patient, or providing critical patient care such as upper airway suctioning, disconnecting the patient from the ventilator.

iii. Preoxygenation (100% O2 for 5 minutes) should be performed via HFNC.

iv. Invasive mechanical ventilation in COVID-19 associated acute hypoxemic respiratory failure and ARDS should be with lower tidal volumes (V.T.) (4 to 8 ml/kg predicted body weight, PBW) and lower inspiratory pressures reaching a plateau pressure (Pplat) < 30 cm of H_2O.

v. Positive end-expiratory pressure (PEEP) must be as high as possible to maintain the driving pressure (Pplat-PEEP) as low as possible (< 14 cm H_2O).

vi. Use of neuromuscular blocking agents (NMBA) should be used as needed to facilitate lung-protective ventilation.

vii. In patients with refractory hypoxemia (PaO2:FiO2 of <150 mm Hg), prone ventilation for > 12 to 16 hours per day and the use of a conservative fluid management strategy for ARDS patients without tissue hypoperfusion are strongly emphasized.

viii. The National Institutes of Health (NIH) Covid-19 Treatment Guidelines Panel recommends against inhaled pulmonary vasodilators such as nitric oxide.

ix. Lung-protective ventilation can also reduce the risk of new or worsening AKI (Acute Kidney Injury) by preventing ventilator-induced hemodynamic effects.

x. ECMO should be considered in carefully selected patients with refractory hypoxemia despite lung-protective ventilation and patients who fail to respond to prone position ventilation.[33]

Management Of COVID-19 Based On The Severity of Illness

Asymptomatic or Presymptomatic Infection

Individuals with a positive SARS-CoV-2 test without any clinical symptoms consistent with COVID-19 should be advised to isolate themselves and monitor clinical symptoms.[34]

Mild Illness

Based on the NIH guidelines, individuals with mild illness is manageable in the ambulatory setting with supportive care and isolation. Laboratory and radiographic evaluation are routinely not indicated. Elderly patients and those with pre-existing conditions should be monitored closely until clinical recovery is achieved.

SARS-CoV-2 neutralizing antibodies such as **REGN-COV2 (casirivimab and imdevimab)** or **bamlanivimab/etesevimab** or **sotrovimab** can be considered for outpatients who are at risk of disease progression with a low threshold to consider hospitalization for closer monitoring. The National Institutes of Health (NIH) Covid-19 Treatment Guidelines Panel recommends against dexamethasone in mild illness.

Moderate Illness

Patients with moderate COVID-19 illness should be hospitalized for close monitoring. Clinicians and healthcare staff should don appropriate personal protective equipment (PPE) while interacting or taking care of the patient. All hospitalized patients should receive supportive care with isotonic fluid resuscitation if volume-depleted, and supplemental oxygen therapy must be initiated if SpO2 and be maintained no higher than 96%. Empirical antibacterial therapy should be started only if there is a suspicion of bacterial infection and should be discontinued as early as possible if not indicated.

Patients with COVID-19 are at risk of developing venous and thromboembolic events and should be maintained on thromboembolic prophylaxis with appropriate anticoagulation. **Remdesivir** and **dexamethasone** can be considered for patients who are hospitalized and require supplemental oxygen.

The National Institutes of Health (NIH) Covid-19 treatment guidelines panel recommends the use of either **remdesivir alone** or **dexamethasone plus remdesivir** or **dexamethasone alone** if combination therapy (remdesivir and dexamethasone) is not available in hospitalized patients who require supplemental oxygen but are not receiving HFNC or NIPPV or IMV or ECMO.

Severe/Critical Illness

Patients with severe/critical COVID-19 illness require hospitalization. Considering that patients with severe COVID-19 are at increased risk of prolonged critical illness and death, discussions regarding care goals, reviewing advanced directives, and identifying surrogate medical decision-makers must be made.

All patients should be maintained on prophylactic anticoagulation, considering COVID-19 is associated with a prothrombotic state. Clinicians and other healthcare staff must wear appropriate PPE that include gowns, gloves, N95 masks, and eye protection when performing aerosol-generating procedures on patients with COVID-19 in the ICU, such as endotracheal intubation, bronchoscopy, tracheostomy, manual ventilation before intubation, physical proning of the patient or providing critical patient care such as nebulization, upper airway suctioning, disconnecting the patient from the ventilator, and noninvasive positive pressure ventilation that may potentially lead to the aerosol generation.

Renal replacement therapy should be considered in renal failure when indicated. Having awake patients self-prone while receiving HFNC can improve oxygenation if endotracheal intubation is not indicated. However, the efficacy of performing this maneuver on awake patients is not clear and more data from clinical trials is needed.

The National Institutes of Health (NIH) Covid-19 Treatment Guidelines Panel strongly recommends using **dexamethasone** in hospitalized patients who require oxygen via noninvasive or invasive ventilation. Combination therapy with **dexamethasone plus remdesivir or baricitinib or tocilizumab in combination with dexamethasone alone** is also recommended in hospitalized patients on HFNC or NIPPV with evidence of disease progression. If corticosteroids cannot be used**, baricitinib plus remdesivir** may be used in non intubated patients.

The National Institutes of Health (NIH) Covid-19 Treatment Guidelines Panel also recommends **tocilizumab** (as a single intravenous dose) in recently hospitalized patients who are exhibiting rapid respiratory decompensation due to COVID-19.Impending respiratory failure should be recognized as early as possible, and endotracheal intubation with IMV must be initiated as described earlier. Vasopressors should be started to maintain mean arterial pressure (MAP) between 60 mmHg and 65 mmHg. Norepinephrine is the preferred initial vasopressor. Empiric antibacterial therapy should be considered if there is a concern for a secondary bacterial infection. Antibiotic use must be reassessed daily for de-escalation, and the duration of the treatment requires evaluation for appropriateness based on the diagnosis.[34]

2- deoxy-D-glucose in the treatment of COVID -19

The drug, named 2-deoxy-D-glucose (2-DG), has been developed jointly by Institute of Nuclear Medicine and Allied Sciences, a lab of the Defence Research and Development Organisation (DRDO), in collaboration with Dr Reddy's Laboratories, Hyderabad.

The Drugs Controller General of India (DCGI) earlier this month granted permission for emergency use of the drug as an adjunct therapy in moderate to severe Covid patients

2-DG is a modified glucose molecule that has been found to have some therapeutic value as an anticancer and antiviral agent. Research on 2-DG goes as far back as 1956, although it hasn’t been approved to treat any other diseases yet. It is currently mostly used in diagnostic testing and research-related activities

Reports claim that this drug selectively accumulates in infected cells and cuts off the energy supply to the virus. Therefore, the virus cannot multiply automatically, which helps in reducing the infection and the viral load gradually. Eventually, the cells recover.

This image, which the government shared in a press release, shows that cell cultures in a laboratory without 2-DG had more viral plaques – clear spots indicating cell damage by the virus – compared to the ones with 2-DG. These studies were conducted at the Centre for Cellular and Molecular Biology, Hyderabad.[35]

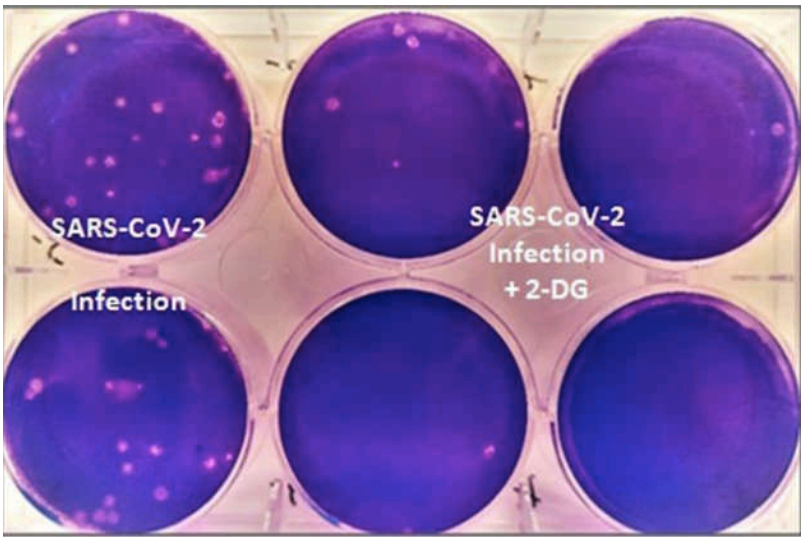


Image of cell cultures in an in vitro study of 2DG.

Courtesy: The Times of India

While these experiments show that 2-DG can inhibit viral growth, they tell us little about its efficacy in humans.[35]

Managing through Timelines

Normally when a virus enters our body and multiplies, our body recognizes the virus and eliminates it through the production of antibodies. Same thing happens with corona virus, so at times, whenever the virus particles increase, antibodies also increases and the interaction between them will lead to release of chemicals known as cytokines. So, the cytokines will result in symptoms like high temperature, cough, sore throat, body pain etc.

Even though the incubation period varies, it is typically around 5 plus or minus 2 days. In the second wave of the disease there is a surge, and no one knows the source of infection. So, onset of first symptoms should be noted. It can be a cough, fever, body ache, sore throat, headache or even diarrhea which is mainly due to irritation from cytokines and virus. It is important to get the precise date. Failure to note the date can lead to serious consequences.
So, by 2nd and 3rd day, symptoms present may go high and by 4th to 5th day the symptoms comes down. So in 80% of the cases, it is the end result of infection. However, in 15-20% cases it takes a dangerous turn. The fever may come back by 6th day, or the symptoms accentuates further. The patient presents with high fever, chest congestion or deep cough. This marks the dangerous turn.

Normally, immune system recognizes viral spike proteins and produces antibodies. However, some cells have protein receptors similar to spike proteins, especially blood vessels

in the lungs, intestine, liver and kidney.so antibodies recognize similar proteins and thus attacks own cells. This will in turn leads to the alteration of smoothness of the internal surface of blood vessels and will lead to the activation of intrinsic pathway of blood coagulation. Because of this clotting mechanism normal blood flow is inhibited in the lungs thus normal air exchange doesn't take place. In some this damage is catastrophic may be due to genetic predilection or environmental factors.

So, once we recognize the dangerous turn i.e., around 5th or 6th day from the onset of symptoms, at which time cytokine shower is starting, steroids can be given inorder to inhibit cytokine storm and the associated effects. So, dexamethasone is considered as the game changer. Along with steroid, which is given for a period of 10 days, anticoagulants are also given to reduce the clotting. Steroid therapy timing is very important because administering in the initial days i.e., during viremia, body's immune system will be lowered, so less of antibodies more of viral replication occurs and accentuates the disease process. So once viremia is over around 6th day steroids are given along with anticoagulant which is administered for a period of 3 weeks as tendency for clotting remains for 3 weeks. Hence most of the patients recovers.

Super-antibodies in the treatment of covid-19

The US Food and Drug Administration (FDA) granted Emergency Use Authorization (EUA) in late May to sotrovimab, providing a new therapeutic weapon in the fight against SARS-CoV-2 and future coronaviruses with pandemic potential.

Sotrovimab traces its roots back to blood drawn in 2013 from an individual who had recovered from the 2003 outbreak of severe acute respiratory syndrome (SARS). In lab studies, sotrovimab seems to maintain its neutralization capacity against all circulating variants of concern, including some of the most worrying versions of the virus, first identified in South Africa, Brazil and India. Several of the leading phase 3 mAb candidates—including Adagio's ADG20, AstraZeneca's AZD7442 and Brii Biosciences' BRII-196 and BRII-198—do as well. But Eli Lilly's two-mAb cocktail is hobbled by escape mutations found in these variants, as is one of the agents, casirivimab, in Regeneron's mAb combination. Many companies then optimized their mAbs by extending half-lives, enhancing neutralizing activity, manipulating constant region (Fc)-mediated effector functions, or applying some combination of these engineering strategies.[36]

6. VACCINATION

Equitable access to safe and effective vaccines is critical to ending the COVID-19 pandemic, so it is hugely encouraging to see so many vaccines proving and going into development. WHO is working tirelessly with partners to develop, manufacture and deploy safe and effective vaccines.

Safe and effective vaccines are a game-changing tool: but for the foreseeable future we must continue wearing masks, cleaning our hands, ensuring good ventilation indoors, physically distancing and avoiding crowds.

Being vaccinated does not mean that we can throw caution to the wind and put ourselves and others at risk, particularly because research is still ongoing into how much vaccines protect not only against disease but also against infection and transmission.

But it's not vaccines that will stop the pandemic, it's vaccination. We must ensure fair and equitable access to vaccines, and ensure every country receives them and can roll them out to protect their people, starting with the most vulnerable.

COVAXIN

The Bharat Biotech COVID-19 Vaccine (COVAXIN^{TM}) is a vaccine with approval for restricted use in emergency situation that may prevent COVID-19. The Central Licensing Authority has granted permission for the sale or distribution of COVAXIN for restricted use in emergency situation in public interest as an abundant precaution, in clinical trial mode.

In phase 1 and phase 2 clinical trials, COVAXIN^{TM} has demonstrated the ability to produce antibodies against COVID-19. However, the clinical efficacy of COVAXIN is yet to be established and it is still being studied in phase 3 clinical trial. Hence, it is important to appreciate that receiving the vaccine does not mean that other precautions related to Covid-19 need not be followed.

The BHARAT BIOTECH COVID-19 VACCINE (COVAXIN^{TM}) includes the following ingredients: COVAXIN^{TM} contains 6µg of whole-virion inactivated SARS-CoV-2 antigen (Strain: NIV-2020-770), and the other inactive ingredients such as aluminum hydroxide gel (250 µg), TLR 7/8 agonist (imidazoquinolinone) 15 µg, 2-phenoxyethanol 2.5

mg, and phosphate buffer saline up to 0.5 ml. The vaccine (COVAXIN^{TM} thus has been developed by using inactivated/killed virus.The BHARAT BIOTECH COVID-19 VACCINE will be given to you as an injection into the deltoid muscle of the upper arm. The BHARAT BIOTECH COVID-19 VACCINE (COVAXIN^{TM}) vaccination series is 2 doses given 4 weeks apart.

The vaccine has an efficacy rate of 81%, preliminary data from its phase 3 trial shows.[37]

Courtesy: bharathbiotech.com

COVISHIELD

The Oxford-AstraZeneca vaccine is being manufactured locally by the Serum Institute of India, the world's largest vaccine manufacturer. The vaccine is made from a weakened version of a common cold virus (known as an adenovirus) from chimpanzees. It has been modified to look more like coronavirus - although it can't cause illness. The COVISHIELD vaccine will be given to you as an intramuscular (IM) injection only, preferably in the deltoid muscle.

The COVISHIELD vaccination course consists of two separate doses of 0.5 ml each. If you receive one dose of the COVISHIELD vaccine, then the second dose should be administered between 4 to 6 weeks after the first dose. However, there is data available for administration of the second dose up to 12 weeks after the first dose from the overseas studies.[38]

Courtesy: The Economic Times

SPUTNIK V

The vaccine, developed by Moscow's Gamaleya Institute, initially generated some controversy after being rolled out before the final trial data had been released.

But scientists say its benefits have now been demonstrated.

It uses a cold-type virus, engineered to be harmless, as a carrier to deliver a small fragment of the coronavirus to the body.

Safely exposing the body to a part of the virus's genetic code in this way allows it to recognise the threat and learn to fight it off, without the risk of becoming ill.

After being vaccinated, the body starts to produce antibodies especially tailored to the coronavirus.This means that the immune system is primed to fight coronavirus when it encounters it for real.It can be stored at temperatures of between 2 and 8C degrees (a standard fridge is roughly 3-5C degrees) making it easier to transport and store.

Unlike other similar vaccines, the Sputnik jab uses two slightly different versions of the vaccine for the first and the second dose - given 21 days apart. They both target the coronavirus's distinctive "spike" but use different vectors - the neutralized virus that carries the spike to the body. The idea is that using two different formulas boosts the immune system even more than using the same version twice - and may give longer-lasting protection.

Courtesy: Anadolu agency

While globally, the Covid vaccines developed so far are to be administered in two doses, this Russian candidate has another variant that has the advantage of being administered in a single dose. The one-dose vaccine, dubbed as **Sputnik Light**, could speed up the pace of vaccination drive in countries like India, inoculating twice as many people at the same time. Sputnik Light was approved for emergency use in Russia in May, with an efficacy rate of 79.4 per cent. Discussions on granting it emergency use approval in India are currently on.

Sputnik V used the SARS-CoV-2's genetic instructions to build the spike protein and stores the information in the double-stranded DNA. The vaccine has been developed from adenoviruses, a kind of virus that causes colds. Researchers added the gene for Covid spike protein to two adenoviruses, engineering them to invade affected cells. The Sputnik V derives inspiration from the adenovirus used to create a vaccine for Ebola by Johnson & Johnson.

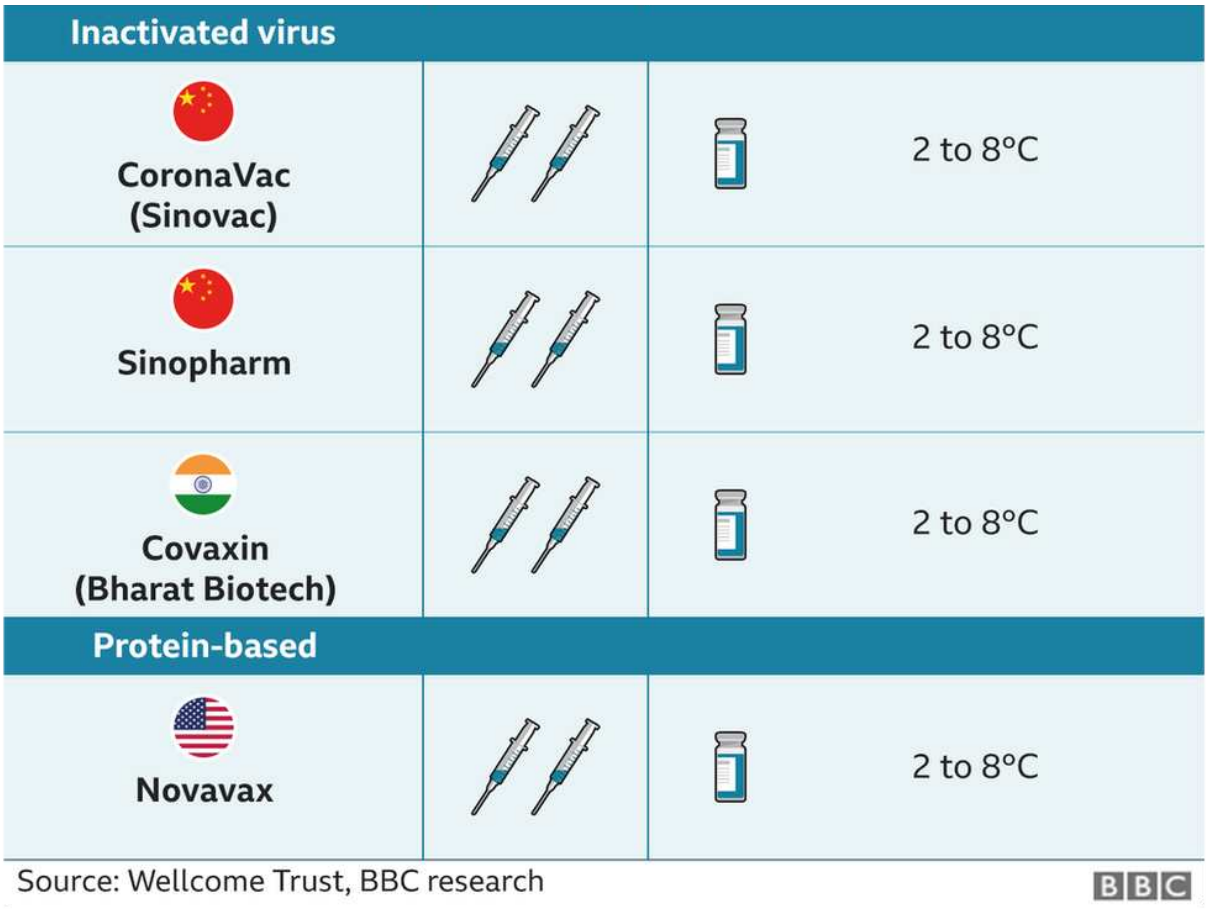
Once injected the adenovirus latches on to the spike proteins engulfing the virus in a bubble travelling to the nucleus of the affected cell where its DNA is stored. The adenovirus is designed in a way that it cannot replicate itself but the gene of the spike protein can be read by the cell and copied into a molecule called messenger RNA, or mRNA. The cells molecules begin assembling spike proteins breaking them into fragments and the adenovirus provokes the immune system to react strongly to these spike proteins as helper T cells trigger antibody generation.

The Sputnik Light also works on a similar design and uses recombinant human adenovirus to carry the code for spike proteins. The vaccine uses a similar design as the J&J which uses human adenovirus serotype number 26.

However, the company is yet to release scientific papers detailing the trial phase of the Sputnik Light vaccine and it is not yet clear as to how long the immunity from the vaccine lasts.

However, the developers have confirmed that the jab is less effective against the South African variant of Covid-19 but claimed that it still offers protection against other mutations than its rivals.

Company	Doses	Storage
RNA		
Pfizer (BioNTech)		-80 to -60°C (6 months) and 2 to 8°C (for up to 5 days)
Moderna		-25 to -15°C (6 months) and 2 to 8°C (for 30 days)
Viral vector		
Oxford-AstraZeneca		2 to 8°C (6 months)
Sputnik V (Gamaleya)		-18.5°C (liquid form) 2 to 8°C (dry form)
Johnson & Johnson (Janssen)		2 to 8°C (3 months)



A single dose of the vaccine induced a robust immune response and was generally well-tolerated. In line with this, in a multiple vaccination regimen (e.g., prime-boost vaccinations) antibodies against the viral vector produced after the prime vaccination can decrease the immunogenicity of booster administrations. Using less common vector serotypes or non-human viral vectors (eg. adenovirus derived from chimpanzees) can help circumvent this immunological conundrum.[39]

Mixing Covaxin and Covishield

On August 7,2021 scientists of the Indian Council of Medical Research (ICMR) made an interesting claim via a preprint paper: that mixing Covaxin and Covishield, the two major vaccines in India's COVID-19 vaccine drive, may not only be safe but may also induce a greater immune response.

The study was led by Pragya Yadav, a scientist at the maximum containment facility of ICMR-National Institute of Virology, Pune. She and her colleagues have described the results of their analysis of antibodies to the novel coronavirus among 18 people who received a first dose of Covishield and, accidentally, a second dose of Covaxin at a vaccination centre in Uttar Pradesh, in May 2021.

The researchers studied the immunological effects of this administrative fiasco. That they hadn't *planned* to examine the effects of a mixed-vaccine schedule makes theirs a serendipitous study, instead of a deliberate experimental trial. The title of their paper is 'Serendipitous COVID-19 vaccine-mix in Uttar Pradesh, India: Safety and immunogenicity assessment of a heterologous regime'.[40]

A good idea to mix?

Using a combination of vaccines as a strategy against COVID-19 is not new. Technically called heterologous prime-boost vaccination, the idea is that, in the short-term, mixed schedules could help overcome shortages of particular vaccines that could in turn affect the rollout of a given country's vaccination programme. And if a combination boosts the immune response and, later, the effectiveness, that would be a major bonus.

In early April 2021, French public health authorities recommended that people younger than 55 years and who had received a primary dose of the Oxford-AstraZeneca vaccine should receive a second dose of one of the mRNA vaccines – either Pfizer-BioNTech or Moderna. They also called for clinical trials on such heterologous, or mixed, schedules and for the state health apparatus to continue its pharmacovigilance.

In July, authorities in Ontario, Canada, backed a similar proposal for a mixed vaccine schedule, and also suggested a first dose of the AstraZeneca vaccine and second dose of an mRNA vaccine for better protection.

In a preprint paper published by *The Lancet* in May 2021, scientists at the University of Oxford had reported a study of such a schedule in the Com-COV clinical trial. This was a phase 2 study with a cohort of 830 participants. Their goal was to find that a heterologous COVID-19 vaccination schedule *didn't* induce lower antibody levels than a homologous

schedule. They succeeded. They also reported more frequent adverse reactions following the second dose of the heterologous schedule – but the reactions were short-lived and minor.

Studies like this are complicated, but the headline results thus far have been encouraging. That is, there is sense in pursuing a heterologous prime-boost vaccination schedule.

This said, unlike the Oxford Com-COV study – a planned, well-designed phase 2 trial – the ICMR study was opportunistic. So before we further unpack the latter, let's remember that the most it is capable of showing us is that the heterologous vaccine schedule didn't seriously compromise the participants' antibody response.[40]

3rd dose of Covid-19 vaccine

Right from the onset of the Covid-19 pandemic, it was evident that some groups are more vulnerable to serious disease—males; those above the age of 65; those having hypertension, diabetes, obesity, chronic underlying renal, cardiac, hepatic or pulmonary disease; those on immunosuppressants; and those on chemotherapy for malignant disease.

It is easy to understand how those with chronic illnesses, organ dysfunction, immunosuppression or malignancy have poor immune responses. This has been shown with other viral infections—Hepatitis B for example. The reasons for the predilection of males, obese subjects and those in the older age group to serious coronavirus infection are not very clear. The virus can cause serious disease even if the inoculum is small, if the immune system of these subgroups is in some way unable to mount an adequate immune response

A study on fully vaccinated healthcare workers published in *The Lancet* throws some light on this important question. They studied the antibody titres against the virus in a large number of fully vaccinated healthcare workers using a standardised automated chemiluminescence assay; they found that the antibody response is significantly lower in precisely those individuals—the older age group, men, those who are obese—who develop severe disease with natural infection.

Those in the older age group, particularly men if they are also obese, will not develop good antibody titres against the virus. It is a common observation during the second wave in India that even subjects who have completed two doses of vaccine are getting infected and that some of them become seriously ill. Men over the age of 65 and obese individuals

with a Body Mass Index of more than 30 constitute a significant proportion of India's population and therefore, the suboptimal immune response in them to vaccination is of public health importance.

In India now, two vaccines are in use: Covishield and Covaxin. The most common vaccine used in India so far is Covishield. It is prepared using a Trojan horse approach—complementary DNA coding for the spike protein, derived from coronavirus RNA, is inserted into a non-replicating chimpanzee adenovirus to prepare the vaccine. Covaxin is an inactivated whole virus vaccine. Both are administered as two doses; Covishield 12 weeks apart and Covaxine 4-6 weeks apart.

Covishield was originally intended to be a single dose vaccine but a second dose was added on as the antibody response to a single dose was not adequate. The problem is that the human immune response is directed not only against the virus-derived spike protein but also against the adenovirus vector. Therefore, a third dose of a vectored vaccine is unlikely to elicit the required degree of immune response and antibody titre because antibodies against the vector virus will inactivate the virus before it can act to produce a booster response. If an inactivated vaccine is used after two doses of a vectored virus vaccine, it is possible that the protective efficacy in these vulnerable subjects may be enhanced. Inactivated vaccines are designed to evoke a broad range of immune responses against several viral antigens, not just the spike protein. Further, we know that mRNA vaccines that elicit a vigorous immune response and high antibody titres are effective against the variants of concern, including the delta virus. A boosted antibody response to a third dose of the vaccine may be expected to work in a similar fashion and be effective against variants of concern.

Taken together, these observations imply that older individuals, particularly men and those with obesity, may need a third dose of the vaccine as a booster in order to achieve adequate levels of protective antibodies. For those who had two doses of a vectored vaccine, the third dose can be an inactivated virus vaccine. For those who had received two doses of an inactivated virus vaccine, a third dose with the same inactivated vaccine may serve as a booster.Bharat Biotech has proposed a booster dose after six months of the second dose.

The Pfizer-BioNTech COVID-19 Vaccine is currently authorized for emergency use in individuals ages 12 and older, and the Moderna COVID-19 Vaccine is authorized for emergency use in individuals ages 18 and older. Both vaccines are administered as a series of

two shots: the Pfizer-BioNTech COVID-19 Vaccine is administered three weeks apart, and the Moderna COVID-19 Vaccine is administered one month apart. The authorizations for these vaccines have been amended to allow for an additional, or third, dose to be administered at least 28 days following the two-dose regimen of the same vaccine to individuals 18 years of age or older (ages 12 or older for Pfizer-BioNTech) who have undergone solid organ transplantation, or who are diagnosed with conditions that are considered to have an equivalent level of immunocompromise**.**

Pfizer CEO Albert Bourla said people will likely need a third dose of a Covid-19 vaccine within 12 months of getting fully vaccinated. He also said it's possible people will need to get vaccinated against the coronavirus annually.[41]

7. INFECTION CONTROL

Infection prevention control (IPC) is a critical and integral part of clinical management of patients and should be initiated at the point of entry of the patient to hospital (OPD or Emergency Department). Standard precautions should always be routinely applied in all areas of health care facilities. Standard precautions include hand hygiene; use of appropriate PPE to avoid direct contact with patients' body fluids, secretions (including respiratory secretions). Standard precautions also include prevention of needle-stick or sharps injury; safe waste management; cleaning and disinfection of equipment and cleaning of the environment. [13]

Infection control to be followed at triage

Give suspect patient a triple layer surgical mask and direct patient to an earmarked and separate area, an isolation room if available. Keep at least 6 feet distance between suspected patients and other patients. Instruct all patients to cover nose and mouth during coughing or sneezing with tissue or flexed elbow for others. Perform hand hygiene after contact with respiratory secretions.

Elements of standard precautions

Apply standard precautions according to risk assessment for all patients, at all times, when providing any diagnostic, clinical care and vaccination services. Standard precautions include hand hygiene and the use of personal protective equipment (PPE) when risk of droplets, aerosols, splashes or in contact with patients' body fluids and secretions (including respiratory secretions). Standard precautions also include appropriate patient placement; prevention of needle- stick or sharps injury; linen management, safe waste management; cleaning and disinfection of equipment; and cleaning of the environment. Best practices for safely managing bio-medical waste should be followed.

Hand hygiene is the best way to prevent the spread of germs in the health care setting and community. Our hands are our main tool for work as health care workers- and they are the key link in the chain of transmission. When using soap and water wash hands for 40-6-secs. Rub hands for 20-30secs while using alcohol-based hand rub. When hands are visibly dirty or contaminated with proteinaceous material, always use soap, running water and single use towel.

All persons with signs and symptoms of a respiratory infection (regardless of presumed cause) must follow respiratory hygiene/cough etiquette:

i. Cover nose and mouth with tissue when coughing or sneezing
ii. Dispose of tissues in the nearest waste receptacle after use immediately
iii. Perform hand hygiene after contact with respiratory secretions and contaminated objects/materials
iv. In absence of tissue/handkerchief patient to be instructed to cover their nose and mouth with arm with elbows flexed during coughing/sneezing
v. Do not spit here and there

Ensure availability of materials for adhering to respiratory hygiene/cough etiquette in waiting areas for patients and visitors:

i. Provide face masks for symptomatic patients.
ii. Provide tissues and no-touch receptacles (i.e. waste container with foot- operated lid or uncovered waste container) for used tissue disposal.
iii. Provide conveniently located dispensers of alcohol-based hand rub.
iv. Provide soap and disposable towels for hand washing where sinks are available.

Principles of PPE use

i. Always perform hand hygiene before and after wearing PPE.
ii. PPE should be available where and when indicated;
    - According to risk
    - In the correct size
iii. Always put PPE on before contact with the patient.
iv. Remove PPE immediately after completing the task and/or leaving the patient care area.
v. Never reuse disposable PPE.
vi. Clean and disinfect reusable PPE between each use.
vii. Change PPE immediately if it becomes soiled/ contaminated or damaged.
viii. PPE should not be adjusted or touched during patient care.
ix. Never touch your face while wearing PPE.

x. if there is concern and/or breach of these practices
   - leave the patient care area when safe to do so
   - properly remove and change the PPE

xi. Always remove PPEs carefully to avoid self-contamination from dirtiest to cleanest areas.[13]

Courtesy: psnetwork.org

Droplet and airborne precautions

As per WHO Current evidence suggests that the virus spreads mainly between people who are in close contact with each other, typically within 1 metre (short-range). A person can be infected when aerosols or droplets containing the virus are inhaled or come directly into contact with the eyes, nose, or mouth.

The virus can also spread in poorly ventilated and/or crowded indoor settings, where people tend to spend longer periods of time in physical proximity. This is because aerosols remain suspended in the air or may travel farther than 1 metre (long-range).

Hence, in all patient care areas, while providing patient care, healthcare worker should wear N-95 mask. In addition, when providing care in close contact with a patient with respiratory symptoms (e.g. coughing or sneezing), use eye protection (face-shield or goggles). Healthcare workers performing aerosol-generating procedures (i.e. open suctioning of respiratory tract, intubation, bronchoscopy, cardiopulmonary resuscitation) use PPE, including gloves, long-sleeved gowns, eye protection, and particulate respirators (N95). Use negative pressure rooms with minimum of 12 air changes per hour for aerosol generating procedures. If not feasible, use well ventilated single rooms using natural or fresh air.

Contact precautions

Contact precautions prevent direct or indirect transmission from contact with contaminated surfaces or equipment (i.e. contact with contaminated oxygen tubing/interfaces). Use PPE (triple layer medical mask or N 95 respirator depending upon the risk assessment, eye protection, gloves and gown) when entering room and take precautions to safely remove PPE when leaving. If possible, use either disposable or dedicated equipment (e.g., stethoscopes, blood pressure cuffs and thermometers). If equipment needs to be shared among patients, clean and disinfect between each patient use. Ensure that health care workers refrain from touching their eyes, nose, and mouth with potentially contaminated gloved or ungloved hands. Avoid contaminating environmental surfaces that are not directly related to patient care (e.g., door handles and light switches). Ensure adequate room ventilation. Avoid movement of patients or transport. Perform hand hygiene.[13]

<u>Oral Antiseptics Used Against Viral Infections</u>

Mouthwashes are widely used solutions for rinsing the mouth, especially before oral surgery, due to their ability to reduce the number of microorganisms in the oral cavity and colony-forming units in dental aerosols the American Dental Association (ADA) and the

Center for Disease Control and Prevention (CDC) have recommended the use of pre-procedural mouthwashes before oral procedures.[42]

Chlorhexidine

CHX is a broad-spectrum antiseptic that acts against Gram-positive and Gram-negative bacteria, aerobes, facultative anaerobes, and fungus by increasing the permeability of the bacterial cell wall, causing its lysis. Evidence indicates an in vitro effect against lipid-enveloped viruses such as influenza A, parainfluenza, herpes virus 1, cytomegalovirus, and hepatitis BCOVID-19 is an enveloped virus, 0.12% CHX gluconate was suggested to have little or no effect against coronaviruses. Yoon et al found SARS-CoV-2 suppression for two hours after using 15 ml 0.12% CHX once, suggesting that its use would be beneficial for the control of COVID-19 transmission.

Courtesy: epharmacy.com

Hydrogen peroxide (H2O2)

H2O2 has been used in dentistry alone or combined with salts since the start of the century. ack of an adverse soft tissue effect was found in many studies of 1%–1.5%
H2O2 used as a daily rinse over two years. An in vitro study found that 3% H2O2 effectively inactivated adenovirus types 3 and 6, adeno-associated virus type 4, rhinoviruses 1A, 1B, and type 7, myxoviruses, influenza A and B, respiratory syncytial virus, strain long and coronavirus strain 229E within 1–30 minutes, discovering that coronaviruses and influenza viruses were the most sensitive. Since SARS-CoV2 is vulnerable to oxidation, preprocedural mouthrinses containing oxidative agents such as 1% H2O2 have been suggested to reduce the salivary viral load.

Cetylpyridinium chloride (CPC)

CPC is a quaternary ammonium compound that is safe for use in humans. Antiviral effect of CPC has been demonstrated in influenza patients, significantly reducing the duration and severity of cough and sore throat. Hypothesis about a possible action over SARS-CoV-2 are based on its lysosomotropic mechanism of action and its ability to destroy viral capsids. These findings indicate that CPC could be effective against other enveloped viruses such as coronaviruses.

Courtesy: medicinestomidnight.co.nz

Iodopovidone

Povidone-iodine (PVP-I) is a water-soluble iodine complex that has been widely used as a pre-surgical skin antiseptic and as a mouthwash. It is typically used in a 1% concentration for mucositis, prophylaxis of oropharyngeal infections, and prevention of ventilator-associated pneumonia. Its antimicrobial action occurs after free iodine dissociates from polyvinylpyrrolidone, then iodine rapidly penetrates microbes to disrupt proteins and oxidises nucleic acid structures causing microbial death.

Previously studies have shown that PVP-I has higher virucidal activity than other commonly used antiseptic agents, including CHX and benzalkonium chloride. It is safe reporting a prevalence of 0.4% allergy cases, does not produce tooth or tongue discoloration or taste disturbances and unlike alcohol based products, can be used when using electricautery. Its effectiveness has been well demonstrated through many in vitro studies against multiple viruses, including SARS-oV, MERS-CoV, and influenza virus A (H1N1) Recent investigations have proposed that 0.23% PVP-I mouthwash for at least 15 seconds before procedures may reduce salivary viral load,44 indicating its use in COVID-19- positive patients.[42]

Courtesy: netmeds.com

Suggested recommendations

Gently gargle for 30 seconds in the oral cavity and 30 seconds in the back of the throat with: 1.5% or 3% H2O2 15 ml; PVP-I, 0.2%, 0.4%, or 0.5% 9ml; 0.12% CHX15 ml; or 0.05% CPC 15 ml.[18]

The use of preprocedural mouth rinses with an oxidative mechanism such as hydrogen peroxide 1% or povidone-iodine 1% has been recommended since these have proven effective against other Coronaviridae family viruses. The effectiveness of povidone-iodine has been well demonstrated through many in vitro studies against multiple viruses, including SARS-CoV, MERS-CoV, and influenza virus A.

Regarding chlorhexidine, however, there is no clear evidence reported regarding its efficacy against SARS CoV2. Few papers document its efficacy against viruses in general and specifically against enveloped viruses, even against coronaviruses, at small concentrations also. However, Peng et al. recommended to avoid chlorhexidine though it was not tested directly against SARS-CoV-2 in their study. Another study by Yoon et al. reported the viral suppression in saliva for 2 h duration. but the recent study showed 0.2% chlorhexidine gluconate appeared most effective compound to achieve the inactivation of virus at merely 30secs of contact time, thus achieving immediate inactivation of the virus. Further, the well-documented property of substantively exhibited by chlorhexidine makes it most appealing to be an effective strategy against SARS CoV 2 transmission in clinical dental settings.[43]

Prevention and treatment of Covid-19 using Homeopathic and Ayurvedic medicines

The Ministry of Ayurveda, Yoga & Naturopathy, Unani, Siddha, Sowa Rigpa and Homoeopathy (AYUSH) of India released two advisories on the preventative and treatment measures that can be taken for the coronavirus epidemic.

One of the advisories was using the homoeopathy drug ***Arsenicum album 30*** as a 'prophylactic medicine' for the prevention of the infection. "As per the Homoeopathy Practices, the following Preventive Management Steps are suggested- The Group of Experts inter-alia has recommended that homoeopathy medicine Arsenicum album30 could be taken as prophylactic medicine against possible Corona virus infections, which has also been advised for prevention of ILI (Influenza Like Illness). It has recommended one doze of Arsenicum album 30, daily in empty stomach for three days. The dose should be repeated after one month by following the same schedule in case Corona virus infections prevail in the community."

Fact-check:

Alt News examined the claims made by the AYUSH Ministry using published scientific evidence at platforms that index scientific articles such as Google Scholar and PUBMED. In the area of drug formulations, clinicians use the research indexed by Google Scholar and PUBMED to verify and seek information regarding new drugs. As a corollary, the absence of scientific research information regarding various drugs in Google Scholar and PUBMED indicates lack of clinical and/or basic scientific research for those drugs. They sci-checked the evidence for Arsenicum album 30 for coronavirus infections, the evidence for any other homoeopathy drug against coronavirus, and Arsenicum album 30 for any other infections.

i. Evidence for Arsenicum album 30 for coronavirus:

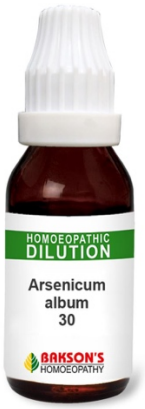
No studies were found that researched the effect of Arsenicum album for coronavirus in humans or animals (in vivo). Also, there were also no studies found in an ex-vivo (outside the animal/human bodies) to study the drug efficacy.

ii. Evidence for any homoeopathy drug for coronavirus:

No studies were found that linked the efficacy of any homoeopathy drug in coronavirus infections.

iii. Evidence for Arsenicum album 30 for any infection:

Searching for evidence in the homoeopathic research for evidence for the drug Arsenicum album 30, only one paper was found, published in British Homoeopathic Journal (Kayne & Rafferty, 1994), that studied its use in neonatal diarrhoea in calves. This study was not only in animals on a different type of infection, but was found to be statistically invalid by Verdier, Öhagen & Alenius in 2003. Thus, Arsenicum album 30 has not been proven or researched scientifically by homoeopaths to cure coronavirus or any other infections in humans.[44]



Arsenicum album 30
Courtesy: IndiaMART

According to Ayurvedic recommendations by the AYUSH ministry, they recommend certain ways to boost the immune systemOne of the best ways to keep COVID-19, or any other infection, at bay, is to strengthen your immune system. The following recommendations may help you do so:

i. Mix a pinch of salt and turmeric in warm water or boil water with Triphala and Yashtimadhu (licorice). Use this concoction to gargle once or twice daily.

ii. Add carom seeds (ajwain), mint leaves or eucalyptus oil to water and do steam inhalation once a day using this concoction.

iii. Apply sesame oil, coconut oil, cow's ghee or medicated oils in your nostrils, especially before going out and after coming back home.

iv. Get six to eight hours of sleep every night, do moderate exercises daily and follow a healthy and balanced diet. Add herbs and spices like ginger, cumin, coriander and holy basil in your diet.

People who have underlying health conditions or are primary contacts or caregivers for those who are COVID-19 positive are considered to be at a high risk of developing severe COVID-19 infection. The following recommendations should be followed by people in these categories:

i. Ashwagandha is a potent herb with antimicrobial properties and is available in the form of both extracts and powders. Take 500mg extract or 1-3g powder, mix it with warm water and consume twice daily for 15 days to a month.

ii. Guduchi or Tinospora cordifolia is another effective Ayurvedic herb and the same amount of extract or powder should be consumed every day after mixing with warm water.

iii. Chyawanprasha is a sticky, dark brown paste made with a mix of a number of Ayurvedic herbs and spices. Consume 10g of it with warm water or milk first thing in the morning on an empty stomach.

Those who have tested positive for COVID-19 but show no symptoms need to take care of their health to ensure they recover quickly and the infection does not get the chance to become worse. For such asymptomatic patients, the AYUSH protocol recommends the consumption of Guduchi (500mg extract or 1-3g powder), a blend of Guduchi and Pippali extract (375mg) and AYUSH 65 (500mg) for 15 days to a month, or as per the recommendations of an Ayurvedic doctor.

Similarly, those with mild COVID-19 symptoms should consume 375mg of the blend of Guduchi and Pippali extract and 500mg dose of AYUSH 65 for the same period of time. This recommendation should be followed only if you have mild symptoms like fever, headache, tiredness, dry cough, sore throat or nasal congestion. Get medical help is you experience breathlessness or hypoxia.

The AYUSH Ministry also recommends that those who are recovering from COVID-19 infection should consume the prescribed dosage of Ashwagandha, Chyawanprasha and

Rasayana Churna (3g, twice daily) to speed up their recovery and avoid post-COVID-19 lung complications, fibrosis, fatigue and other issues.[45]

Steam Inhalation – an Acceptable Method to Prevent Corona?

A viral post that is doing rounds on the internet says, "If you want to stay safe and kill the coronavirus, which could be hiding inside your nose, then you should start taking steam which will reach to your nose and kill the virus. At 50 degree Celsius, the coronavirus becomes disabled. At 60 degree Celsius, the virus becomes weak, and at 70-degree Celsius, the virus dies completely. The post further reads, "COVID-19 can be killed by inhaling steam from the nose or mouth, eliminating the coronavirus. If all the people started a steam drive campaign for a week, the pandemic will soon end. However, neither World Health Organization (WHO) nor U.S. Centers for Disease Control and Prevention (CDC) recommend this treatment for preventing coronavirus. CDC representative told Reuters that the steam inhalation practice is risky and there is no scientific proof that it can prevent coronavirus. Adding further to the information, the representative said that this steam inhalation is a risky process and can even cause burn injury. On the other hand, several doctors have also warned against steam inhalation and said that in such cases when a person has covid, it could worsen their situation as it could lead to asthma symptoms.

The doctors have suggested that social distancing, masks, washing hands, and sanitizing hands at proper intervals are the only thing to fight with coronavirus.[46]

The Difference Between Droplet and Airborne Transmission

Humans produce droplets in various ways (e.g., sneezing, coughing, singing) and these droplets vary in size. Large droplets (> 5 μm) comprise most of the volume of expelled respiratory droplets and they tend to fall rapidly to the ground. Droplets smaller than 5 μm are referred to as droplet nuclei and may remain suspended in the air for significant periods of time and move with air currents.

Respiratory viruses, including COVID-19 viruses are usually transported in large particle droplets. As enveloped viruses, they are usually not viable in small droplet-nuclei. Droplet transmission occurs when bacteria or viruses travel on relatively large respiratory droplets that people sneeze, cough, or exhale. They travel only short distances (usually less than 2 meters) before settling. These droplets may be loaded with infectious particles and can infect another person if the bacteria/viruses contact their eyes, nose or mouth. They may also fall on surfaces and then be transferred onto someone's hand who then rubs their eyes, nose or mouth.Airborne transmission occurs when bacteria or viruses travel in droplet nuclei that become aerosolized. Healthy people can inhale the infectious droplet nuclei into their lungs.

COVID-19 is primarily transmitted from person-to-person through respiratory droplets. These droplets are released when someone with COVID-19 sneezes, coughs, or talks. Infectious droplets can land in the mouths or noses of people who are nearby or possibly be inhaled into the lungs. A physical distance of at least 1 meter (3 ft) between persons is recommended by the World Health Organization (WHO) to avoid infection,1 whereas CDC recommends maintaining a physical distance of at least 1.8 meters (6ft) between persons.

Respiratory droplets can land on hands, objects, or surfaces around the person when they cough or talk, and people can then become infected with COVID-19 from touching hands, objects or surfaces with droplets and then touching their eyes, nose, or mouth. Recent data suggest that there can be transmission of COVID-19 through droplets of those with mild symptoms or those who do not feel ill2. Current data do not support long range aerosol transmission of SARS-CoV-2, such as seen with measles or tuberculosis. Short-range inhalation of aerosols is a possibility for COVID-19, as with many respiratory pathogens. However, this cannot easily be distinguished from "droplet" transmission based on epidemiologic patterns. Short-range transmission is a possibility particularly in crowded medical wards and inadequately ventilated spaces. Certain procedures in health facilities can generate fine aerosols and should be avoided whenever possible.[47]

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