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REVIEW

# Overview of coronavirus - Understanding origin, pathogenesis, clinical features & diagnostic approach to COVID-19 in Indian scenario

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## Abstract

In early December 2019, an outbreak of novel coronavirus (2019-nCoV) or the severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) as it is now called, occurred in Wuhan City, Hubei Province, China. On January 30, 2020 the World Health Organization declared the outbreak as a Public Health Emergency of International Concern. Vaccines to prevent human coronavirus infections are not yet available. Coronavirus disease 2019 (COVID-19) spreads primarily when people are in close contact with small droplets produced by an infected person, identified as "super spreaders". COVID-19 can be fatal among high-risk groups patients >60 yr. The envelope spike "S" protein receptor binding domain of SARS-CoV-2 use host receptor angiotensin-converting enzyme 2 (ACE2) to enter the cells of airway epithelium and alveolar type 2 (AT2) pneumocytes, and pulmonary cells. The most common clinical features are fever (80-90%), cough (60-80%) and breathlessness (18-46%). The other symptoms include myalgia, sore throat, loss of taste and smell, headache, nausea, vomiting and diarrhoea. Infection control practices to be followed stringently by Health Care Workers (HCW). Standard precautions to be maintained. Specimen to be packed in triple container packing. Cold temperature to be maintained during transport and storage. Laboratory tests of COVID-19 are broadly categorized into two methods: (1) Nucleic acid based assay: RT-PCR, TrueNAT, CBNAAT & (2) Immunoassay: Can be broadly divided into 2 types: Antigen based assay: Rapid antigen test, Antibody based assay: enzyme-linked immunosorbent assay (ELISA).

***Keywords:*** Coronavirus; COVID-19; laboratory diagnosis; SARS-CoV-2

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## Background

In early December 2019, an outbreak of novel coronavirus (2019-nCoV) or the severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) as it is now called, occurred in Wuhan City, Hubei Province, China. On January 30, 2020 the World Health Organization (WHO) declared the outbreak as a Public Health Emergency of International Concern [1]. On March 11, the WHO declared a pandemic at this point there were an estimated 1,18,000 cases in 114 countries, resulting in 4,291 reported deaths [2].

Coronaviruses (CoVs) represent a group of viruses mostly affecting human beings through zoonotic transmission. In the past two decades, this is the third instance of the emergence of a novel coronavirus, after severe acute respiratory syndrome (SARS) in 2003 and Middle East respiratory syndrome coronavirus (MERSCoV) in 2012 [3, 4]. As of mid-April 2020, the infection has spread to over 185 countries, infected more than two million people and resulted in over 127,000 deaths globally [5].

## Origin

Coronaviruses are present in nature in environment since ancient times. Most recent common ancestor (MRCA) of all coronaviruses is estimated to have existed 8000 BCE, some models suggest 55 million years. A long term coevolution with bat and avian species [6]. alphacoronavirus exist since 2400 BCE, betacoronavirus exist since 3300 BCE, gammacoronavirus exist since 2900 BCE and deltacoronavirus exist since 3000 BCE.

The natural reservoirs are warm blooded flying vertebrates, are the coronavirus gene pool (with bats alphacoronavirus and betacoronavirus, with birds gammacoronavirus, and deltacoronavirus) as shown in Table 1. The large number and global range of bat and avian species that host viruses has enabled extensive evolution and dissemination [7]. Many human coronaviruses have their origin in bats and associated with other animals as shown in Table 2.

**Table 1:** Classification of coronaviruses.

| *S.No* | *Genus* | *Type species* | *Species* |
|---|---|---|---|
| 1 | Alphacoronavirus | Alphacoronavirus 1 (TGEV) | Alphacoronavirus 1, human coronavirus 229E, human coronavirus NL63, miniopterus bat coronavirus 1, miniopterus bat coronavirus HKU8, porcine epidemic diarrhea virus, rhinolophus bat coronavirus HKU2, scotophilus bat coronavirus 512. |
| 2 | Betacorona virus | Murine coronavirus (MHV) | Betacoronavirus 1 (Bovine coronavirus, human coronavirus OC43), hedgehog coronavirus 1, human coronavirus HKU1, middle east respiratory syndrome-related coronavirus (MERS), murine coronavirus, pipistrellus bat coronavirus HKU5, rousettus bat coronavirus HKU9, severe acute respiratory syndrome-related oronavirus (SARS-CoV, SARS-CoV-2), tylonycteris bat coronavirus HKU4. |
| 3 | Gammacorona virus | Avian coronavirus (IBV) | Avian coronavirus, beluga whale coronavirus SW1 |
| 4 | Deltacorona virus | Bulbul coronavirus (HKU1) | Bulbul coronavirus HKU11, porcine coronavirus HKU15 |

Coronaviruses are a group of related RNA viruses that cause diseases in mammals and birds. In humans, these viruses cause respiratory tract infections that can range from mild to lethal.

They cause mild illnesses like the common cold (which is also caused by other viruses, predominantly rhinoviruses), while more lethal varieties can cause SARS, MERS, and COVID-19.

There are as yet no vaccines or antiviral drugs to prevent or treat human coronavirus infections. Members of coronaviruses, a few members with SARS were identified as “super spreaders” each appeared to have infected more than 10 contacts.

The name "coronavirus" is derived from Latin corona, meaning "crown" [8]. The name was coined by June Almeida and David Tyrrell who first observed and

studied human coronaviruses [9]. The name refers to the characteristic appearance of virions (the infective form of the virus) by electron microscopy, which have a fringe of large, bulbous surface projections (viral spike peplomers) creating an image of the solar corona or halo [9, 10] (Figure 1).

**Table 2:** Association of human coronavirus with animals.

| *S.No* | *Name of human coronavirus* | *Animal* | *Time period* | *Important Information* |
|---|---|---|---|---|
| 1 | HCV NL 63 | Bat | 1190- 1449 CE | Shares a common ancestor with bat coronavirus (ARCoV-2) |
| 2 | HCV 229E | Bat | 1686-1800 CE | Shares a common ancestor with bat coronavirus (Ghana Grp 1 Bt CoV) |
| 3 | MERS CoV emerged in humans from bats & camels | Bat<br>Camels<br>(intermediate host) | MERS diverged several centuries ago | |
| 4 | SARS CoV | Bat<br>Civets<br>(intermediate host) | | SARS related coronaviruses evolved in bats for a long time. The ancestors of SARS CoV first infected leaf-nose bats (genus- Hipposideridae), subsequently, they spread to horse-shoe bats (genus-Rhinolophidae), then to Asian palm civets, finally to humans |

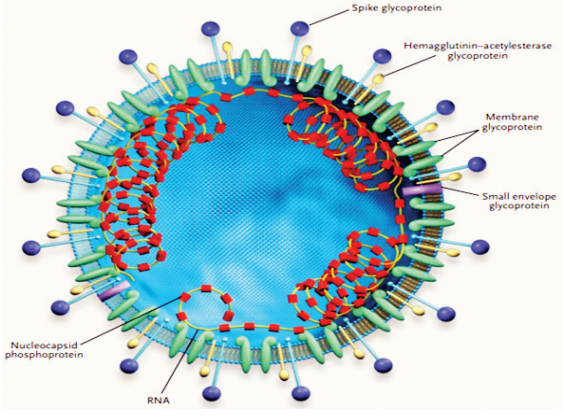


**Figure 1:** Structure of coronavirus.

In 1930, infectious bronchitis virus (IBV) causing acute respiratory infection in domesticated chicken was first discovered. In 1931 and 1940, two more coronaviruses mouse hepatitis virus (MHV) and transmissible gastroenteritis virus (TGEV), were isolated [11].

## Structure and composition

Coronaviruses are enveloped 120-160 nm particles that contain an unsegmented genome of single stranded positive sense RNA (27-32 kbp). It is the largest genome among RNA viruses. The envelope is a bilipid layer. Isolated genomic RNA is infectious. The helical nucleocapsid is 9-11 nm in diameter. There are 20 nm long club or petal-shaped projections that are widely spaced on the outer surface of envelope suggestive of solar corona. The viral structural proteins include N protein (50-60 kDa), M protein (20-35 kDa). They both serve as a matrix protein embedded in envelope lipid bilayer and interacting with a nucleocapsid and the spike (S 180-220 kDa) glycoprotein that makes a petal shaped peplomers. The ratio of E:S:M in the lipid bilayer is approximately 1:20:300 [12]. On average a coronavirus particle has 74 surface spikes. A subset of coronaviruses (specifically the members of betacoronavirus subgroup A) also have a shorter spike-like surface protein called hemagglutinin esterase (HE) [13].

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 [14, 15]. The lipid bilayer envelope, membrane proteins, and nucleocapsid protect the virus when it is outside the host cell [16].

## Genome

Coronaviruses contain a positive-sense, single-stranded RNA genome. The genome size for

coronaviruses ranges from 26.4 to 31.7 kilobases [17]. The genome size is one of the largest among RNA viruses. The genome has a 5' methylated cap and a 3' polyadenylated tail [14].

The genome organization for a coronavirus is 5′-leader-UTR-replicase/transcriptase-spike and 1b, which occupy the first two-thirds of the genome, encode the replicase-transcriptase polyprotein (pp1ab). The replicase-transcriptase polyprotein self cleaves to form 16 nonstructural proteins (nsp1–nsp16) [14].

The later reading frames encode the four major structural proteins: spike, envelope, membrane, and nucleocapsid. Inter spread between these reading frames are the reading frames for the accessory proteins. The number of accessory proteins and their function is unique depending on the specific coronavirus [14] **(**Figure 2).

Betacoronaviruses genome organization; The betacoronavirus for human (SARS-CoV-2, SARS-CoV and MERS-CoV) genome comprises of the 50 -untranslated region (50- UTR), open reading frame (ORFs) 1a/b (green box) encoding non-structural proteins (nsp) for replication, structural proteins including spike (blue box), envelop (maroon box), membrane (pink box), and nucleocapsid (cyan box) proteins, accessory proteins (light gray boxes) such as orf 3, 6, 7a, 7b, 8 and 9b in the SARS-CoV-2 genome, and the 30- untranslated region (30 -UTR). The dotted underlined in red are the protein which shows key variation between SARS-CoV-2 and SARS-CoV. Table 3 shows various characteristics of coronavirus strains MERS-CoV, SARS-CoV, SARS-CoV-2 [18-22].

**Table 3:** Characteristics of coronavirus strains MERS-CoV, SARS-CoV & SARS-CoV-2.

| Virus | MERS-CoV (Middle east respiratory syndrome coronavirus) | SARS-CoV (Severe acute respiratory coronavirus) | SARS-CoV2 (Severe acute respiratory coronavirus 2) |
|---|---|---|---|
| Disease | Middle east respiratory syndrome | Severe acute respiratory coronavirus | Coronavirus infectious disease 2019 (COVID-19) |
| Year of outbreak | 2012, 2015, 2018 | 2002-2004 | 2019-2020 |
| | Epidemic | Epidemic | Pandemic |
| Spread | Saudi Arabia, UAE, Republic of Korea | China, Hongkong, Chinese Taipei, Singapore, Hanoi in Vietnam, Toronto | World wide |
| Epidemiology | | | |
| Date of 1st identified case | June 2012 | November 2002 | December 2019 |
| Location of 1st identified case | Jeddah city, Hejaz region, Saudi Arabia | Shunde district of city Foshan, Guangdong province, China | Wuhan city, Hubei Province, China |
| Average age | 56 | 44 | 56 |
| Sex ratio (M:F) | 3.3:1 | 0.8:1 | 1.6:1 |
| Confirmed case | 2494 | 8096 | 48,46,427 (India)* |
| Deaths | 858 | 774 | 79,754 (India)* |
| Case fatality rate | 37% | 9.2% | 1.70% (India)** |
| Symptoms | | | |
| Fever | 98 % | 99-100 % | 87.9 |
| Dry cough | 47 % | 29-75 % | 67.7 % |
| Dyspnea | 72 % | 40-42 % | 18.6 % |
| Diarrhoea | 26 % | 20-25 % | 3.7 % |
| Sore throat | 21 % | 13-25 % | 13.9 % |
| Ventilatory support | 24.5 % | 14-20 % | 4.1 % |

* updated on 14 Sep 2020; ** updated on 7 Sep 2020

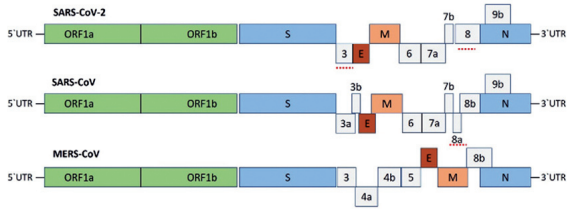


**Figure 2:** Genomic organization of betacoronaviruses.

## Transmission

COVID-19 spreads primarily when people are in close contact, small droplets produced by an infected person (symptomatic or not) coughing, sneezing, talking, or singing [23]. The WHO recommends 1 metre (3 ft) of social distance, the U.S. CDC recommends 2 metres (6 ft).

People are most infectious when they show symptoms (even mild or non-specific symptoms), but may be infectious for up to two days before symptoms appear (pre-symptomatic transmission). They remain infectious an estimated seven to twelve days in moderate cases and an average of two weeks in severe cases [24].

The surface on which droplet fall is present, when touched, contaminated surfaces and then their eyes, nose or mouth with unwashed hands is a method of transmission. On surfaces the amount of virus can be detected for up to four hours on copper, up to one day on cardboard, and up to three days on plastic (polypropylene) and stainless steel.

Surfaces are easily decontaminated with household disinfectants which kill the virus outside the human body or on the hands. Disinfectants or bleach are not a treatment for COVID-19, and cause health problems when not used properly, such as when used inside the human body.

Estimates of the number of people infected by one person with COVID-19 (the R0) have varied widely. The WHO's initial estimates of the R0 were 1.4-2.5 (average 1.95), to be higher at 3.28 and the median R0 to be 2.79 [25].

It has acquired a 'super spreader' status, wherein cases have been implicated through asymptomatic transmission or in some where the mode of transmission is unexplainable.

## Risk factor

COVID-19 can be fatal among high-risk groups patients >60 yr as opposed to young adults or pediatric population. It was estimated that the highest mortality rates were seen among patients above 80 yr of age (14.8 %) whereas patients with no prior comorbid conditions had a case fatality rate of 0.9 %.

The following conditions might be at an increased risk for severe illness from COVID-19: Asthma (moderate-to-severe), cerebrovascular disease (affects blood vessels and blood supply to the brain), cystic fibrosis, hypertension or high blood pressure, Immunocompromised state (weakened immune system) from blood or bone marrow transplant, immune deficiencies, HIV, use of corticosteroids, or use of other immune weakening medicines. Neurologic conditions, such as dementia, liver disease, pregnancy, pulmonary fibrosis (having damaged or scarred lung tissues), smoking, thalasemia & type 1 diabetes mellitus [26].

## Pathogenesis

The exact mechanism of SARS-CoV-2 is not known. The envelope spike "S" protein receptor binding domain of SARS-CoV-2 is similar to SARS-CoV. SARS-CoV-2 use host receptor angiotensin-converting enzyme 2 (ACE2) to enter the cells of airway epithelium and alveolar type 2 (AT2) pneumocytes, and pulmonary cells [27].

The envelope spike "S" proteins is of two types, S1 & S2. S1 is responsible for receptor binding and S2 is responsible for cell membrane fusion [28].

Immediately after binding to the receptive receptor, SARS-CoV-2 enters host cells where they encounter the innate immune response. To infect the new host, SARS-CoV-2 must be able to inhibit or evade host innate immune signaling. However, it is largely unknown how SARSCoV-2 manages to evade immune response and drive pathogenesis.

To inhibit viral replication and dissemination, the host immune system responds by mediating inflammation and antiviral cellular activity. However, excessive immune responses together with lytic effects of the virus on host cells will result in pathogenesis. These patients are reported to have

higher plasma levels of proinflammatory cytokines including IL1, IL-2, IL7, TNF alpha, GSCF, MCP1 suggestive of a cytokine storm. It infects older males and rarely children [29].

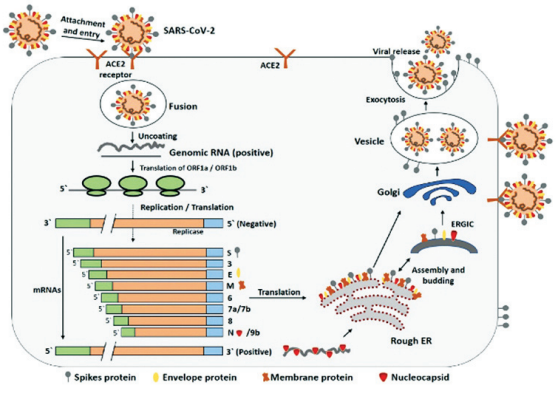


**Figure 3:** Coronavirus replication cycle.

Coronavirus binds to host cell specific receptor glycoprotein or glycans by the spike protein. Penetration and uncoating occur by S protein mediated fusion of the viral envelope with the plasma membrane or endosomal membranes (Figure 3).

Gene 1 of viral genomic RNA is translated into a polyprotein, which is processed to yield transcriptase-replicase complex.

Genomic RNA is used as a template to synthesize negative stranded RNAs, which are used to synthesize full length genomic RNA and subgenomic mRNAs. Each mRNA is translated to yield only the protein encoded from 5'end of the mRNA including non-structural proteins.

The N protein and newly synthesized genomic RNA assemble to form helical nucleocapsids.

Membrane glycoprotein M is inserted in the endoplasmic reticulum (ER) and anchored in the Golgi apparatus.

Nucleocapsid (N plus genomic RNA) binds to M protein at the budding compartment (ERGIC). E and M proteins interact to trigger the budding virions, enclosing nucleocapsid.

S and HE glycoproteins are glycosylated and trimerized, associate with M protein, and are incorporated into maturing virus particles.

Virions are released by exocytosis like fusion of vesicles with plasma memebrane. Virions may remain adsorbed to plasma membranes of infected cells. The entire cycle of coronavirus replication occurs in the cytoplasm [30].

## Clinical features

The incubation period of COVID-19 is 3-14 days (mean duration of 5-7 days). The most common clinical features with which the patient present are fever (80-90%), cough (60-80%) and breathlessness (18-46%). Other symptom that can be associated with chief clinical features include myalgia or fatigue, sore throat, running nose, nasal congestion, headache, nausea, vomiting and diarrhoea [31]. Diarrhoea and vomiting could be an important signs of Covid-19 in children. CNS manifestations include dizziness, headache, impaired consciousness, acute cerebrovascular disease, ataxia, and seizure. Deep vein thrombosis is a rare presentation.

There are warning signs also that need immediate clinical intervention. Fever and upper respiratory symptoms lasting for >5 days and any of the following conditions such as breathlessness/respiratory rate >24/min oxygen saturation (SpO2) <95% in room air, fatigue with heart rate of >110/beats per minute, systolic blood pressure <90mm Hg [32].

## Diagnostic approach to COVID-19 in Indian scenario

*Advisory on strategy for COVID-19 testing in India:* (VersionVI, 4th September 2020) Indian Council of Medical Research. Department of Health Research, Ministry of Health and Family Welfare, Government of India [33].

### A. Routine surveillance in containment zones and screening at points of entry

### Choice of Test (in order of priority)

i. Rapid antigen test (RAT)

ii. RT-PCR or TrueNat or CBNAAT

1. All symptomatic (ILI symptoms) cases including health care workers and frontline workers.
2. All asymptomatic direct and high-risk contacts (in family and workplace, elderly ≥65 years of age, immunocompromised, those with co-morbidities etc.,) of a

laboratory confirmed case to be tested once between day 5 and day 10 of coming into contact.

3. All asymptomatic high-risk individuals (elderly ≥65 years of age, those with comorbidities etc.,) in containment zones.

**B. Routine surveillance in non-containment areas:**

**Choice of test (in order of priority**):

i. RT-PCR or TrueNat or CBNAAT

ii. Rapid antigen tet (RAT)*

1. All symptomatic (ILI symptoms) individuals with history of international travel in the last 14 days.
2. All symptomatic (ILI symptoms) contacts of laboratory confirmed case.
3. All symptomatic (ILI symptoms) health care workers / frontline workers involved in containment and mitigation activities.
4. All symptomatic ILI cases among returnees and migrants within 7 days of illness.
5. *All asymptomatic high-risk contacts (contact in family and workplace, elderly ≥65 years of age, those with co-morbidities etc., (RAT is recommended as per the first choice in order of priority).

**C. In hospital settings:**

**Choice of test (in order of priority**):

i. RT-PCR or TrueNat or CBNAAT

ii. Rapid antigen tet (RAT)

1. All patients of Severe Acute Respiratory Infection (SARI).
2. All symptomatic (ILI symptoms) patients presenting in a healthcare setting.
3. Asymptomatic high-risk patients who are hospitalized or seeking immediate hospitalization such as immunocompromised individuals, patients diagnosed with malignant disease, transplant patients, patients with chronic co-morbidities, elderly ≥65 years.
4. Asymptomatic patients undergoing surgical/ no-surgical invasive procedures (not to be tested more than once a week during hospital stay).
5. All pregnant women in /near labor who are hospitalized for delivery.

Points to be noted:

- No emergency procedure (including deliveries) should be delayed for lack of test. However, sample can be sent for testing if indicated as above (1-13), simultaneously.
- Pregnant women should not be referred for a lack of testing facility. All arrangements should be made and transfer samples to facilities.
- Mothers who test positive for COVI-19 should be advised to wear a mask and undertake frequent handwashing while handling their baby for 14 days. They should also be advised on breast cleaning before feeding the neonate. These measures are likely to reduce transmission of COVID-19 to their babies.

6. All symptomatic neonates presenting with acute respiratory /sepsis like illness. (Features suggestive of acute respiratory illness in a neonate are respiratory distress or apnoea with or without cough, with or without fever. Neonates may also manifest with only non-respiratory symptoms like fever, lethargy, poor feeding, seizures or diarrhoea).
7. Patient presenting with atypical manifestations [stroke, encephalitis, hemoptysis, pulmonary embolism, acute coronary symptoms, Gullian Barre syndrome, multiple organ dysfunction syndrome, progressive gastrointestinal symptoms, Kawasaki Disease (in pediatric age group)] based on the discretion of the treating physician.

## D. Testing on demand (State Governments to decide simplified modalities)

1. All individuals undertaking travel to countries, Indian states mandating a negative COVID-19 test at point of entry.
2. All individuals who wish to get themselves tested.

## Laboratory diagnosis

The laboratory diagnosis depends on the duration of illness, collection, transportation, storage of sample & tests performed.

A laboratory request form (ICMR) is filled by the treating physician with patient demographic details, date, time and anatomical site of the sample collection, clinical history, symptoms and test required need to be mentioned.

Specimen collection: A personal protective equipment (PPE) is required to be worn by the healthcare worker before collecting a sample.

*Specimen packaging:* It should be safely packed in triple container packing and sent to Molecular Biology laboratory while maintaining the cold temperature. The packaging system consist of 3 layers as follows:

1. The original sample should be packed, labeled and marked with UN3373 for Category B, Biological Substances. Orientation label, Handle with care
2. Standard triple packing for Category B to be followed
3. Samples to be sent on icebox using cold ice pack (dry ice preferred).

**Table 4:** Information of specimen collection, storage & transport [34, 35].

| *S. No* | *Specimen* | *Collection material* | *Storage & transport* |
|---|---|---|---|
| 1 | Nasopharyngeal swab Oropharyngeal swab | Dacron or polyster flocked swab in viral transport medium (VTM) in a sterile leak proof container | Refrigerate at 2-8°C upto 5 days. If >5days freeze at -70°C and ship on dry ice |
| 2 | Sputum | Sterile, wide mouthed, leak proof container | Refrigerate at 2-8°C upto 48 hours. If >48 hours freeze at -70°C and ship on dry ice. |
| 3 | Bronchoalveolar lavage | 2-3 ml in a sterile leak proof container | Refrigerate at 2-8°C upto 48 hours. If >48 hours freeze at -70°C and ship on dry ice. |
| 4 | Endotracheal or nasopharyngeal aspirate | 2-3 ml in a sterile leak proof container | Refrigerate at 2-8°C upto 48 hours. If >48 hours freeze at -70°C and ship on dry ice. |
| 5 | Venous blood | Serum separator tube (For antibody testing) | Store upright for at least 30 minutes after collection. Refrigerate and ship at 2-8°C upto 5 days. |

*Note:* Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit PCR testing.

*Note:* If both NP and OP swabs are collected, they should be combined in a single tube to maximize test sensitivity and limit use of testing resources.

Biosafety measures to be followed by HCW in a COVID-19 testing laboratory [36].

Non sterile gloves (single use), gown, eye protection, N95 mask are used for specimen collection, specimen receipt & accession and specimen testing (non-propagative & propagative).

## Laboratory Testing methods of COVID-19 [37]

I) Nucleic acid based assay: RT-PCR, TrueNAT, CBNAAT

II) Immunoassay: Can be broadly divided into 2 types.

1. Antigen based assay: Rapid antigen test (RAT).
2. Antibody based assay: Enzyme-linked immunosorbent assay (ELISA)

## Processing of the sample in Molecular Laboratory

Safety of health care workers in the laboratory is important. PPE along with N95 mask has to be worn. The samples are processed in a Biological Safety Cabinet Level 2 (BSC L2) in a negative pressure room. Nucleic acid extraction step is done in BSL 2.

## Molecular assay -RT PCR

Current testing modalities for laboratory diagnosis of COVID-19 by real time PCR. The targets for molecular assay for SARS-CoV-2 are mentioned below.

1. Initial Screening RT PCR involves detection of 'E' gene (coding for SARS-CoV-2 viral envelope).
2. Confirmation of samples positive in screening PCR involves detection of one of the following gene targets:

   a. RdRp gene (coding for SARS-CoV-2 RNA dependent RNA polymerase).

   b. ORF gene (coding for SARS-CoV-2 Open Reading Frame).

   c. N gene (coding for SARS-CoV-2 Nucleocapsid).

Table 5 mentions information regarding RT-PCR protocols for detection of SARCoV-2 proposed by ICMR & WHO [38].

**Table 5:** RT-PCR protocols for detection of SARCoV-2 proposed by ICMR & WHO.

| *S.No* | *Name of the organization* | *Molecular method of testing* | *Detects* | *Steps* | *Target genes* |
|---|---|---|---|---|---|
| 1 | ICMR | RT-PCR | 1. Sarbecovirus<br>2. SARS CoV-2 | Multiplex PCR screening for E gene<br>And RdRp/ N gene | E gene<br>RdRp |
| 2 | US CDC | RT-PCR | 1. Universal detection of SARS-like<br>β coronavirus<br>2. Specific detection of SARS CoV-2 | Three separate RT-PCR target the N gene.<br>1st primer set detects all βcoronavirus<br>2 sets detect specific for SARS CoV-2.<br>All 3 positive. Report -Presumptive positive for SAR CoV-2 | N gene |
| 3 | The Charite' algorithm.<br>Berlin, Germany | RT-PCR | 1. Subgenus Sarbecovirus (SARS CoV & SARS CoV-2)<br>2. βcoronavirus | Detects E gene.<br>RdRp genes of subgenus.<br>Both assays should be positive | E gene<br>RdRp |
| 4 | University of Hong Kong,<br>Li Ka Shing, Faculty of Medicine | RT-PCR | 1. Sarbecovirus<br>2. SARS CoV-2 | 1. Screening test for detection of N gene<br>2. Confirmatory test for detection of Orf1b | N gene<br>Orf1b |

**Table 6:** A comparative table of RT-PCR, TrueNat & CBNAAT [39-41].

| | *RT-PCR* | *TrueNat* | *CBNAAT* |
|---|---|---|---|
| Principle | Nucleic Acid Amplification | Chip-based real time PCR test.<br>2 steps: "E" gene screening for suspected COVID-19. Confirmatory test Rd/Rp enzyme found in virus. | Automated qualitative cartridge based nucleic acid amplification |
| Specimen | Respiratory specimen | Respiratory specimen | Respiratory specimen |
| Turn around time | 4-5 hours | Less than 1 hour | Less than 1 hour |
| Number of test | Depends on the capacity of machine (30-450) | Single test at a time (Quadrinat4 tests at a time) | Single test at a time |
| Infrastructure & Laboratory safety | Bio safety level-2<br>Appropriate PPE | Bio safety level-2*<br>Appropriate PPE | Bio safety level-2<br>Appropriate PPE |
| Advantage | Most widely used screening & confirmatory test<br>High sensitivity. High specificity | Quick, portable.<br>Cheap and affordable.<br>Can be used in<br>Public health centre.<br>Reagents do not require extreme temperatures. | Closed nature platform and minimum sample handling, minimum biosafety hazard |
| Disadvantage | Cumbersome procedure.<br>Performed by skilled analyst. | 1 to 4 samples can be tested at a time (maximum 24-48/day). | At the same time many samples cannot be tested.<br>Limited availability |

*Note: ICMR - The sample is collected in viral lysis buffer & hence biosafety and biosecurity requirements for use of TrueNat machine is minimal.

Rapid point-of-care(PoC) Antigen Detection Test (Standard Q COVID-19 Ag Kit) [42, 43]

This test use a small, portable, lateral flow assay that can be performed at point of care. Naopharyngeal samples can be tested. It produces colored lines to indicate positive or negative results. It has moderate sensitivity but high specificity.

Most COVID-19 antigen tests target the 'spike protein' that are present on the surface of the coronavirus. A nasopharyngeal swab is collected (Figure 4). The swab is then inserted into an extraction buffer tube, stir the swab more than 5 times and then removed while squeezing the sides of the tube to extract the liquid from the swab. The nozzle cap should be pressed tightly onto the tube. Three drops of extracted specimen should be added into the specimen well of the test device and results should be read within 15-30 minutes [44]. Report test result as positive or negative, as per Algorithm (Figure 5).

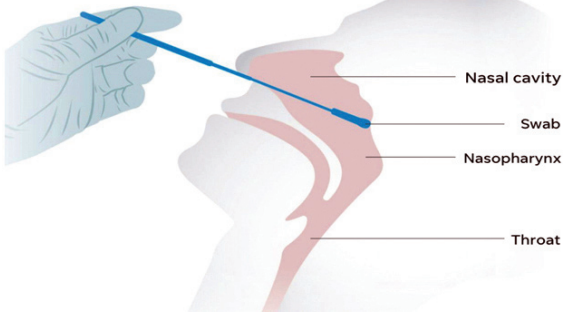


**Figure 4:** Collection of nasopharyngeal swab.

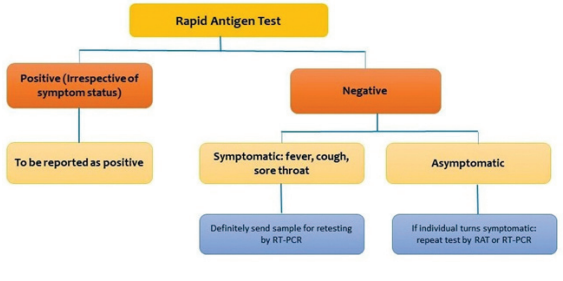


**Figure 5**: Algorithm for COVID-19 test interpretation using rapid antigen point-of-care test. .

- All positive and negative result should be entered into the ICMR portal on a real time basis after performing the antigen test
- Result of samples subjected to RT-PCR should be entered after the RT-PCR results are available

## Advantages of antigen testing

Rapid detection of the result. It reduces the load of RT-PCR. Antigen tests are also inexpensive compared to RT-PCR.

## Limitations of antigen testing

An antigen test can only reveal whether a person is currently infected with SARS-CoV-2. Before or after the infection has passed, antigens won't be present.

Since antigen testing doesn't involve any processes of amplifying the virus or its genetic material, a swab sample may have too little antigen to be detected. This could produce a false negative result. Accuracy is the single largest problem with antigen tests, which are much less sensitive than RT-PCR as a diagnostic tool.

The other validated and approved kit is COVID-19 antigen lateral test device (Labcare Diagnostics) supplied by MyLab Discovery Solutions.

## Antibody test

ELISA is an immunological assay commonly used to measure antibodies, antigens in human serum or plasma. It is generally carried out in 96 well plates, allowing multiple samples to be measured in a single experiment. They have high sensitivity and specificity. ELISA's require sophisticated equipment and skilled technicians to perform the tests [45].

In a press release, ICMR advises states to conduct sero-survey to measure coronavirus exposure in the population using IgG ELISA test on May 30, 2020.

IgG antibodies generally start appearing after two weeks of onset of infection, once the individual has recovered after infection and last for several months. Therefore, the IgG test is not useful for detecting acute infection but indicates episode of SARS-CoV-2 infection in the past. However, detection of antibodies is useful in the following situations:

1. To understand the proportion of population exposed to SARS-CoV-2 infection including asymptomatic individuals.
2. In high risk or vulnerable populations (health care workers, frontline workers, immunocompromised individuals, individuals in containment zones etc.,) to know who has been infected in the past and has now recovered.

Antigen detection test for SARS CoV-2 are directed against Nucleocapsid (N) or Spike (S1 & S2) of the virus protein. Spike protein S1 is specific.

Antibody detection tests Ig G, Ig M & Ig A produced against the N or S viral proteins. The appearance and disappearance of various viral markers by antigen & antibody detection tests are mentioned in Table 7 & Figure 6.

**Table 7:** Viral markers detected in antigen & antibody detection test.

| | *Appear* | *Maximum* | *Disappear/ Remain in blood* |
|---|---|---|---|
| SARS CoV-2 RNA & antigen | 0-5 days | 12-14 days | Disappear after 28 days |
| Ig M | 7-10 days | 14 days | Disappear after 21 days |
| Ig G | 14 days | 21 days | Persist in blood for long periods |

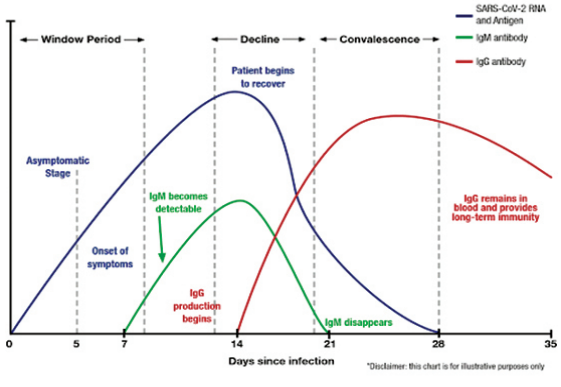


**Figure 6:** Viral markers detected in antigen & antibody detection test.

## Conclusion

In the present challenging situation, it is of primary importance to limit the spread of infection from person to person. The practise of using a mask, maintaining the social distance, cough etiquette are of primary importance. In a hospital setup, maintaining the hospital infection control practices can prevent the transmission of infection from patient to patient as well as patient to health care workers. Use of PPE, surface disinfection and disinfection of instruments appropriately are the important preventive measures. The vaccine has not been developed yet and further research is required. Hence strict infection prevention and control practices and important.

## Limitations

An early diagnosis play an important role in isolating the patient and initiation of treatment. Point of care antigen detection testing (PoC) would be valuable at field level for containment of the disease but antigen tests are much less sensitive than RT-PCR as a diagnostic tool.

Although real-time PCR is the gold standard test for detecting cases of COVID-19, they need skilled personnel and specific biosafety, biosecurity precautions. They are expensive and contamination is a major concern. To minimize and detect contamination of samples in an analytical run, this need properly designed Molecular Laboratory which cannot be performed at district level laboratory.

## Future work

Because of the potential for reemergence of SARS, it is important to move forward with research in diagnostics, therapeutics and vaccines for SARS CoV-2. The research to gain knowledge in the field of (1) determinants of virulence & pathogenesis, (2) mechanisms of genome recombination & mutation & (3) development of stable attenuated viruses for vaccine production.

## Conflicts of interest

Authors declare no conflicts of interest.

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