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ORIGINAL RESEARCH ARTICLE



## Laboratory testing for COVID-19 in suspected human cases and the impact of corona virus pandemic on the global health system

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### ABSTRACT

COVID-19, coronavirus (SARS-CoV-2) infection has become pandemic after first appearing in Wuhan, China in December 2019. It destroyed the life of millions of people throughout the different parts of Europe, America, Asia and others in the world. Various groups of scientists throughout the globe have claimed on trialing for the corona vaccine and for finding out suitable medication for the treatment of COVID-19. No vaccine or medicines are successfully reported to short out the issue for saving the valuable life of human beings, till date. The mechanism of SARS-CoV-2 infection and organ invasion are not understood and it creates difficulty in clinical diagnosis and treatment of corona patients. The pathogenic mechanism of SARS-CoV-2 infection is not very much clear and it may invade multiple organ systems of respiratory, digestive and hematological in a confirmed case. The impact of corona virus outbreak on the global and Indian health systems is also reviewed herewith.

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### GRAPHICAL ABSTRACT



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### INTRODUCTION

Many coronaviruses can infect humans, the worldwide endemic human coronaviruses HCoV-229E, HCoV-NL63, HCoV-HKU1 and HCoV-OC43 that tend to cause mild respiratory disease, and the zoonotic Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome corona-

virus (SARS-CoV) that have a higher case fatality rate. In Wuhan, China on December 2019, patients with a novel coronavirus were identified (Zhu *et al.*, 2020). Initially it was named 2019 novel coronavirus (2019-nCoV), the virus has now been named SARS-CoV-2 by the International Committee of Taxonomy of Viruses (ICTV). This virus can cause the disease named coronavirus disease 2019 (COVID-19) (Gorbalenya *et al.*, 2020;

Chen et al., 2020; Han et al., 2020; Ye et al., 2020; Chavez et al., 2020; Law, 2020; Adelodun et al., 2020).

Novel Coronavirus 2019 (SARS-CoV-2) infection out broke and spread to the world from December 2019. On January 30, 2020, SARS-CoV-2 infection was declared as a global health emergency by World Health Organization (WHO). The mechanism of SARS-CoV-2 infection and organ invasion are unclear, which directly leads to difficulties and blindness in clinical diagnosis and treatment. The pathogenic mechanism of SARS-CoV-2 infection is still unclear. Current evidences indicate that it can invade multiple organ systems, including the respiratory system, digestive system and hematological system (Peng et al., 2019).

The suspected COVID-19 cases may be as follows: (i) all symptomatic individuals who have undertaken international travel in the last 14 days or (ii) all symptomatic contacts of laboratory confirmed cases or (iii) all symptomatic healthcare personnel (HCP) or (iv) all admitted patients with Influenza like illness (ILI) (fever and cough and/or shortness of breath) or (v) asymptomatic direct and high risk contacts of a positive case (should be tested once between day 5 and day 14 after contact) Symptomatic refers to fever/cough/shortness of breath Sore throat, loss of smell. Direct and high-risk contacts include those who live in the same household with a confirmed case and health workers who examined a confirmed case (Angela, 2020). Confirmed case: A person with positive laboratory report of COVID-19 infection, irrespective of clinical signs and symptoms. Many of the most common symptoms of novel coronavirus disease (COVID-19) are similar to those of common flu or cold (Table 1). So, it is also suggested knowing which common symptoms of flu or cold are not symptoms of COVID-19. COVID-19 infection seldom causes a runny nose. Rhinorrhea ("runny nose") is not a symptom of COVID-19 and nasal congestion is reported only by 4.8% of patients.

The most common COVID-19 symptoms are: fever (88%), dry-cough (68%), fatigue (38%), thick sputum production (34%), shortness of breath (19%), arthromyalgia (15%), sore throat (14%), headache (13.6%), chills (11%), nausea/vomiting (5%), nasal congestion (4.8%), diarrhea (3.7%). Beware of patients with gastrointestinal symptoms. Nausea / vomiting and / or diarrhoea can be present in about 9% of cases. These symptoms have so far been one of the most frequent causes of omission or diagnostic delays. Vital signs measurements (do not forget respiratory rate, please) and blood gas analysis in ambient air, if SpO<sub>2</sub> <94%, at triage or as soon as possible, are essential to correctly assess patients coming to the emergency room. PO<sub>2</sub> <60 for the diagnosis of respiratory failure cannot be relied always. The P / F, especially in young subjects should be calculated. "COVID-19 profile" can be defined for the rapid order entry of blood tests, including the following tests: blood count, C-RP, creatinine, blood glucose, albumin, AST ALT, bilirubin, pneumococcal and legionella urinary agents, PT-INR, troponin and procalcitonin. Chest X-rays have limited sensitivity in early stages of COVID-19 pneumonia. CT scan is more sensitive, but raises logistical problems. If ultrasounds competencies are available, chest US can be used, but the US probes should be

disinfected after contact with every COVID-19 suspected patient. Monolateral lung infiltrates do not exclude COVID-19. They have been described in 25% of cases. The most common reported laboratory abnormalities in COVID-19 patients are: Lymphopenia (35-75%), increased C-RP (75-93%), LDH (27-92%), ESR (up to 85% of cases), hypoalbuminemia (50-98%) and anemia (4150%). Data from a systematic revision of literature.

The following negative prognostic factors have been reported: leukocytosis, neutrophilia, increased procalcitonin, LDH, AST, ALT, total bilirubin, creatinine, troponin, d-dimer, PT and hypoalbuminemia, lymphopenia. Even thrombocytopenia is associated with severe disease. Severe lymphopenia and lymphocytes count fall during the first 4 days since hospital admissions have been associated with a higher mortality. Increased white blood cell count, neutrophil count and procalcitonin could reflect bacterial superinfection, while increased d-dimer and PT a diffuse intravascular coagulation (DIC), reported in up to 75% of patients who died. History of smoking, respiratory failure, maximum body temperature on admission C, albuminemia <4 mg/dl would be risk factors for disease progression (severe or critical disease/death (Han et al., 2020). During epidemics it is important to avoid availability bias that means diagnose of all infections due to epidemic agents. WHO recommends investigating other pathogens, as co-infections have been reported. Disease severity stratification should be used for the choice of the treatment setting (home, ordinary, sub-intensive or intensive care unit). WHO distinguishes 6 clinical syndromes associated with COVID-19: uncomplicated disease, mild pneumonia, severe pneumonia, ARDS, sepsis and septic shock. Patients with uncomplicated upper respiratory tract viral infection may have non-specific symptoms such as fever, cough, sore throat, nasal congestion, malaise, headache, muscle pain or malaise. These patients do not have any signs of dehydration, sepsis or shortness of breath and can be treated at home.

Criteria for Intensive Care access should be collectively discussed and defined for each patient in advance involving the medical team and patient/family members, just as any decision to limit treatment should be collegial, motivated, shared with patient/family members and documented in medical records. The factors to be considered in such a decision are: age, functional status, comorbidity, advanced treatment provisions already expressed, availability of resources and eventual discussion with colleagues with proven experience. COVID-19 can lead to a significant increase in the need for ICU beds and a tricky imbalance between need and availability, so uncomfortable ethical issues can arise. Clear criteria and early assessment are essential to avoid hasty and inappropriate decisions.

For suspected COVID 19 patients, testing for COVID-19 19 to be done as per the ICMR guidelines, which state that the testing is to be limited to the following: a) For all asymptomatic individuals who have undertaken international travel in the last 14 days - (i) They should stay in home quarantine for 14 days, ii) They should be tested only if they become symptomatic (fever, cough, difficulty in breathing), iii) All family members living with a confirmed case should be home quarantined. (b) All sympto-

**Table 1.** COVID-19 patient classification.

Travel History/ Contact History	No International travel/ Contact History	Travel/ Contact History more than 14 days	Travel/ Contact History less than 14 days	Direct Contact with Confirmed COVID 19 Case
Fever	98.6 <sup>0</sup> F - 100 <sup>0</sup> F	100 <sup>0</sup> F - 102 <sup>0</sup> F	102 <sup>0</sup> F - 104 <sup>0</sup> F	>104 <sup>0</sup> F
Breathing difficulty	No (You can easily hold your breath for 10 seconds)	Mild (Breathless while climbing steps)	Moderate (Breathless while walking on level ground)	Severe (Breathless even while sitting)
Body Pain	No	Mild	Moderate	Severe
Fatigue/ Weakness	No	Mild	Moderate	Severe
Sore Throat	No	Mild	Moderate	Severe
Cough	No	Mild	Moderate	Severe
Diarrhea	1 - 2 episodes in last 24 hrs	3 to 5 episodes in last 24 hrs	5 to 7 episodes in last 24 hrs	More than 7 episodes in last 24 hrs
Other Medical Conditions	None	High BP	High BP + Diabetes Mellitus, chronic lung condition	Reduced Immunity
Status in Last 48 hrs	Improved	No Change	Worsened	Worsened a Lot
Age	15 to 50 Yrs	5 to 15 Yrs	0 to 5 Yrs	>50 Yrs

matic contacts of laboratory confirmed cases. (c) All symptomatic health care workers. (d) All hospitalized patients with Severe Acute Respiratory Illness (fever and cough and / or shortness of breath). (e) Asymptomatic direct and high-risk contacts of a confirmed case should be tested once between day 5 and day 14 of coming in his/her contact. The objective of this research is to investigate on laboratory testing for COVID-19 in suspected human cases and to extend the study on the impact of COVID-19 pandemic with the global and Indian health system.

## MATERIALS AND METHODS

### Laboratory design and facilities (Level 3)

The laboratory must be separated from the areas that are hospitable unrestricted traffic flow within the building. Additional separation could also be achieved by placing the laboratory at the blind end of a corridor, or constructing a partition and door or access through an anteroom (e.g., a double-door entry or basic laboratory - grade 2), describing a selected area designed to take care of the pressure differential between the laboratory and its adjacent space. Biological safety cabinets should be sited away from walking areas and out of crosscurrents from doors and ventilation system. An autoclave for the decontamination of contaminated waste should be available. Backflow-precaution devices must be fitted to the water supply. Vacuum lines should be protected with liquid disinfectant traps and HEPA filters, or their equivalent. Surfaces of walls, floors and ceilings should be water-resistant and straightforward to wash. A hand-washing station with hands-free controls should be provided near each entrance. There must be a controlled ventilation that maintains a directional airflow into the laboratory room. A visual monitor with or without alarm(s) should be installed in order that staff can in the least times make sure that proper directional airflow into the laboratory room is maintained. Air could also be high-efficiency particulate air (HEPA) filtered, reconditioned and recirculated within that laboratory. All HEPA filters must be installed during a manner that allows gaseous decontamination and testing.

### Sample collection

Area for Sample Collection shall be carried out in a designated Negative pressure isolation room There shall be independent air handling facility through use of exhaust fans and appropriate HEPA filter. The personal shall wear entire PPE while collecting sample.

### Sample type

Sample types are the following: Essential Samples: (a) Throat swab (oropharyngeal swab) (b) Nasal swab (Nasopharyngeal swab) These shall be Dacron or Polyester flocced swabs placed in the same viral transport medium (c) EDTA Blood for Plasma Sample (d) SST for Serum sample.

### Other preferred samples

Bronchoalveolar lavage in a sterile container Tracheal aspirate in a sterile container Nasopharyngeal aspirate or nasal wash in sterile container Sputum (well coughed out from the lower respiratory tract) in sterile container. For transport samples for viral detection, use double packing system containing antifungal and antibiotic supplements. Avoid repeated freezing and thawing of specimens. Priority specimens. Other specimens need to be sent as per the clinical condition of the patient.

### Optimal timing

Within 3 days of symptom onset and no later than 7 days. Preferably prior to initiation of antimicrobial chemoprophylaxis or therapy Laboratory testing guiding principles for patients who meet the suspect case definition? The decision to test should be based on clinical and epidemiological factors and linked to an assessment of the likelihood of infection. PCR testing of asymptomatic or mildly symptomatic contacts can be considered in the assessment of individuals who have had contact with a COVID-19 case. Screening protocols should be adapted to the local situation. The case definitions are being regularly reviewed and updated as new information becomes available.

For the WHO suspected case definition sees: Global Surveillance for human infection with coronavirus disease (COVID-2019). Rapid collection and testing of appropriate specimens from patients meeting the suspected case definition for COVID-19 is a priority for clinical management and outbreak control and should be guided by a laboratory expert. Suspected cases should be screened for the virus with nucleic acid amplification tests (NAAT), such as RT-PCR case management requires, patients should be tested for other respiratory pathogens using routine laboratory procedures, as recommended in local management guidelines for community-acquired pneumonia. Additional testing should not delay testing for COVID-19. As co-infections can occur, all patients that meet the suspected case definition should be tested for COVID-19 virus no matter whether another respiratory pathogen is found. In an early study in Wuhan, the mean time period for COVID-19 was 5.2 days among 425 cases, though it varies widely between individuals. Virus shedding patterns are not yet well understood and further investigations are needed to better understand the timing, compartmentalization, and quantity of viral shedding to inform optimal specimen collection. Although respiratory samples have the greatest yield, the virus can be detected in other specimens, including stool and blood (Ye et al., 2020). Laboratory testing for COVID-19 virus Laboratories undertaking testing for COVID-19 virus should adhere strictly to appropriate bio-safety & good laboratory practices. Nucleic acid amplification tests (NAAT) for COVID-19 virus. Nucleic acid amplification tests (NAAT) for COVID-19 virus Routine confirmation of cases of COVID-19 is predicated on detection of unique sequences of virus RNA by NAAT like real-time reverse transcription polymerase chain reaction (rRT-PCR) with confirmation by nucleic acid sequencing if necessary. The viral genes targeted till now include the N, E, S and RdRP genes. Examples of protocols used may be found here. RNA extraction should be wiped out a biosafety cabinet during a BSL-2 or equivalent facility. Heat treatment of samples before RNA extraction isn't recommended.

Laboratory confirmation of cases by NAAT in areas with no known COVID-19 virus circulation to think about a case as laboratory-confirmed by NAAT in a neighbourhood with no COVID-19 virus circulation, one of the following conditions need to be met: A positive result for at least two different targets on the COVID-19 virus genome, of which at least one target is preferably specific for COVID-19 virus using a validated assay (as at present no other SARS-like coronaviruses are circulating in the human population it can be debated whether it has to be COVID-19 or SARS-like coronavirus specific); OR - One positive NAAT result for the presence of betacoronavirus, and COVID-19 virus further identified by sequencing partial or whole genome of the virus as long because the sequence target is larger or different from the amplicon probed in the NAAT assay used.

When there are conflicting results, the patient should be re-sampled and, if appropriate, sequencing of the virus from the first specimen or of an amplicon generated from an appropriate NAAT assay, different from the NAAT assay initially used, should

be obtained to supply a reliable test result. Laboratories are encouraged to seek confirmation of any surprising results in an international reference laboratory. Laboratory confirmed case by NAAT in areas with established COVID-19 virus circulation In areas where COVID-19 virus is widely spread an easier algorithm could be adopted during which for instance screening by rRT-PCR of a single discriminatory target is considered sufficient. One or more negative results don't rule out the likelihood of COVID-19 viral infection. A number of factors could lead to a negative result in an infected individual, including: poor quality of the specimen, containing little patient material (as a control, consider determining whether there is sufficient human DNA in the sample by including a human target in the PCR testing) - the specimen was collected late or very early in the infection - the specimen was not handled and shipped appropriately - technical reasons inherent in the test, e.g., virus mutation or PCR inhibition. If a negative result is obtained from a patient with a high suspicion for COVID-19 infection, only on upper respiratory tract specimens, additional specimens, including from the lower respiratory tract should be collected and tested if possible. Each NAAT run should include both external and internal controls, and laboratories are encouraged to participate in external quality assessment schemes when they become available. It is also recommended to laboratories who order their own primers and probes to perform entry testing/validation on functionality and potential contaminants (Chavez et al., 2020).

#### Viral sequencing

In addition to providing information of the presence of the virus, regular sequencing of a percentage of specimens from clinical cases can be helpful to monitor for viral gene mutations that might affect the performance of medical countermeasures, including diagnostic tests. Virus whole genome sequencing also can inform molecular epidemiology studies.

#### Viral culture

Virus isolation is not recommended as a routine diagnostic procedure (Law, 2020).

## RESULTS AND DISCUSSION

Research toward improved detection of COVID-19 virus many aspects of the virus and diseases are still not understood. More research is required to understand and control the COVID-19 disease.

#### Dynamic of immunological response

Disease severity in various populations, e.g., by age. The relationship between viral concentration and disease severity. The duration of shedding, and relation to clinical setting (e.g., Recovery occurs with viral clearing, or shedding persists despite clinical improvement). Development and validation of useful serological assays. Comparative studies of available molecular and serological assays. Adequate percentage of positive cases that requires sequencing to monitor mutations that might affect the performance of molecular tests.

**Table 2.** The impacts of COVID-19 in some hospitals of different countries.

Start	End	Country	Urban area	Shortages, comments and sources
Late January	Late February	China	Wuhan, Hubei	Turn away most patients due to health care facility capacity overwhelmed (WHO, Geneva, Jan 2020). Ventilators.
February 2020		Italy	Lombardy	Ventilator, hospital beds. Population density, trade relations with China, health care reforms lowering relevant medical capacities and management mistakes blamed for the crisis' severity (WHO, February 2020).
March 2020		France	Alsace	Due to PPE shortages, French citizens had to collect face masks and 3D-print face shields in order to donate them to healthcare workers for better safety. The French government had to shuttle patients from less affected regions (GISAID, February 2020).
April 2020	Mid may	Massachusetts	Worcester	Two field hospitals built to care for lighter patients (United Nations, April 2020).
Late April 2020		Brazil	Manaus	3 times more deaths than usual (The Japan Times, April 2020).
May 2020		Peru	Loreto	Oxygen therapy shortage (The Japan Times, April 2020).
Early May 2020		Mexico	Tijuana	Bed shortage, medical staff sick (Li and Joyu, 2020).
Early May 2020		Mexico	Mexico city	Bed shortage (Li and Joyu, 2020), 11,000 medical staff tested positive (Kurmanaev <i>et al.</i> , 2020).
Early May 2020		India	Delhi	Bed shortage (Euronews, May 2020).
11 May 2020		India	Mumbai	Bed shortage (NBC Boston, May 2020; Anatoly <i>et al.</i> , 2020)
22 May 2020		Boarder of USA -Mexico	Southern California	80 Bed non-COVID temporary systems, many patients of US-citizen residing in nearby Mexican town, turned away locally (Azam and Daniel, 2020).

### Impact of COVID-19 epidemic on health Systems

The health system got priorities because of emerging of COVID-19 globally. But the capacities and the services of the hospitals to the communities were hindered due to lock down. The disturbance of the supply of logistics greatly affected the health services. Due to this the availability of medical and healthcare facility were hindered for the patients with acute or chronic ailments to access standard health care facility. Children, women and elderly people were more suffered because of lack of treatment. The impact of the COVID-19 pandemic resisted from the treatment of infectious diseases. The epidemic has decreased the household income of many of the families for a longer period of time. The mortality rate of child will be more in the year 2020 in compare to without COVID-19. The COVID-19 created the cancelation of all polio and measles vaccination campaigns throughout the globe that stopped the prevention from these diseases in many countries (Peng *et al.*, 2019). Also, children are prohibiting their growth from malnutrition as the schools are closed. So, in many cases they are not getting proper food. Continuous support of health system is required for the Patients with HIV/AIDS and TB with regular intake of medicines. Disturbance in the supply of medication is very much detrimental to the health and hygiene of the patients. Again, the patients who need regular administration of cancer therapies face serious negative impacts on COVID-19. The emergency services like accidents and immediate operations are not available in many hospitals. COVID-19 affected the patients with acute coronary syndromes, tumour resection, cancer care, psychiatric disease etc. Fear of COVID-19 infection also prevented people from taking support of hospitals. The COVID-19 has caused health care infrastructures to become overwhelmed which resulted more deaths and/or increased

crisis in the local health care systems. The impacts of COVID-19 in some hospitals of different countries are reflected in Table 2 (Government of India, March, 2020).

### COVID-19 impact on the Indian health system

An acute health crisis has been observed in India in last few months because of emerging of COVID-19 in India.

(i) A large number of children might already have missed vital immunizations. (ii) Many adults might have missed life-saving medical treatment (like chemotherapy) as the as the lockdown came into force. (iii) The basic health services in the lockdown period were disrupted. There were curtailed immunization schedules, restricted inpatient/outpatient and emergency treatment for infectious diseases, lowered laboratory investigations and less access to psychiatric treatment. (iv) Maternal health care services have been severely curtailed.

The healthcare impact on COVID-19 pandemic of India has been studied using a stochastic mathematical model by (Chatterjee *et al.*, 2020). They have studied the Age-stratified impact of COVID-19 on hospitalization, ICU admission and fatality in India (Table 3).

### Impact of health care in rural India

The rural area of India faces a chronic shortage of medical professionals which is detrimental to the rural healthcare system, in terms of availability of quality healthcare there (Free Malaysia Today, 2020). The poor infrastructure and lack of coordination between the line departments make it difficult to tackle with COVID-19. The health system in rural areas of India is not adequate to contain COVID-19 because of the shortage of doctors, hospitals, beds and necessary equipments (Reuters News Agency, 2020).

**Table 3.** Age-stratified impact of COVID-19 on hospitalization, ICU admission and fatality in India (Source: Chatterjee et al., 2020).

Age group (years)	Indian demographic distribution (%) (Hindustan Times, 2020)	Hospitalization rate (The Guardian, 2020)	ICU admission rate (% of hospitalization rate) (The Guardian, 2020)	Infection fatality rate (The Guardian, 2020)
0-9	18.4	0.1	5	0.002
10-19	18.8	0.3	5	0.006
20-29	17.5	1.2	5	0.03
30-39	15.3	3.2	5	0.08
40-49	11.7	4.9	6.3	0.15
50-59	9	10.2	12.2	0.6
60-69	5.8	16.6	27.4	2.2
70-79	2.7	24.3	43.2	5.1
80+	0.8	27.3	70.9	9.3

The government hospital beds in rural India are 3.2 per 10,000 people (Free Malayasia Today, 2020). Many states have a significantly lower number of rural beds than the national average. The rural area of Uttar Pradesh has 2.5 beds per 10,000 people. Rajasthan and Jharkhand have 2.4 and 2.3 rural beds, respectively (Free Malayasia Today, 2020). Maharashtra, which has the highest number of COVID-19 patients, has 2.0 beds per 10,000 populations and Bihar has 0.6 beds per 10,000. These states of India have also shortage of specialist doctors (Free Malayasia Today, 2020). Thus, the hospitals of rural India are facing serious problems for treating COVID-19 cases. This is also creating negative impacts to the non-COVID patients who need emergency or routine treatments.

### Conclusion

The corona virus and the associated disease are still not properly understood by the doctors and the researchers. An adequate knowledge and guidance are required to overcome from the cope of the fatal disease of COVID-19. A lot of work is required on the prevention and treatment of corona virus. Invention of corona vaccine is the urgent need of the hour. The scientists of CSIR and DST research labs of India should be actively involved in finding out the medicine and vaccine of COVID-19. People should be more careful for maintaining social distances and improving self immunity for the prevention from COVID-19. The Government should keep the supply chain and logistics open for the prevention and treatment of COVID-19. COVID-19 epidemic has significant economic impact on laboratories/health care institutions (such as, laboratory of radiology with markedly decreased imaging case volumes). Therefore, it is challenge to the health care institutions to survive in a constrained economic environment as the pandemic ensues.

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