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Strengthening of Molecular Diagnosis of SARS-CoV-2 / COVID-19 with a Special Focus on India

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Abstract

Severe acute respiratory syndrome corona virus-2 (SARS-CoV-2), a novel coronavirus initially reported in Wuhan, China, is the causative agent of coronavirus disease (COVID-19) pandemic. Symptoms of the disease comprise of fever, tiredness, dry cough, aches and pains, nasal congestion, runny nose, sore throat, diarrhoea and pneumonia at the late stage. SARS-CoV-2 has severely crippled the healthcare system and has caused huge economic losses. Following the outbreak, the SARS-CoV-2 was recognized timely and its genome was sequenced, leading to the development of real-time polymerase chain reaction assays for its detection in clinical samples collected from suspected cases. The management of the pandemic is limited by a number of misconceptions and insufficient information about laboratory testing for SARS-CoV-2 to confirm the disease. This includes a lack of awareness about procedures for the collection, transport, testing, and handling of biological samples for COVID diagnosis. This article provides an overview of the current laboratory diagnostic methods with a purpose to provide information and guidance to laboratories, stakeholders, broader community and especially public health professionals involved in laboratory testing for SARS-CoV-2.

Keywords: COVID-19, SARS-CoV-2, molecular diagnosis, RT-PCR, prevention, control

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INTRODUCTION

On March 11, 2020, the World Health Organization (WHO) announced the COVID-19 outbreak as pandemic since the number of cases were rapidly increasing worldwide apart from rising disease outbreaks in China[1]. In December 2019, cases of "pneumonia of unknown etiology" were reported in Wuhan (Central China) related to Huanan (Southern China) Seafood Wholesale Market, as an ill health with no causative pathogen, characterized by fever (≥38°C), pneumonia diagnosed by radiological imaging, low or normal white-cell count or low lymphocyte count and no progress in health condition following 3-5 days of antimicrobial treatment. Later, novel coronavirus (2019-nCoV) was identified which is now referred to as SARS-CoV-2 causing COVID-19[2]. The disease started spreading rapidly worldwide and WHO declared the COVID-19 as pandemic disease on March 11, 2019. As of May 11, 2020, confirmed cases, recovered cases and deaths worldwide due to COVID-19 were nearly 4.20 million, 1.5 million and 0.30 million, respectively. The disease epicenter first shifted to Europe, contributing the very high number of new cases and deaths outside of China initially and then to North America contributing the highest number of cases[3].

According to situation reports by the WHO, Region of the Americas, the African Region and Eastern Mediterranean Region, have also reported cases of COVID-19[4]. India reported its 1st case of COVID-19 on January 30, 2020, in the southern coastal state of Kerala in a student who had a travel history to Wuhan, China, followed by two similar cases on February 2 and 3, 2020. On March 2, 2020, two new cases were reported, one each in New Delhi and Hyderabad[5]. Thereafter, the number of cases increased due to a large number of tourists from Italy and other parts of the world, and recently rapid transmission and spread occurred within the country. As of May 11, 2020, nearly 67,000 confirmed cases and 2,200 deaths were accounted due to COVID-19 in India

Following the recognition of Wuhan as epicenter of the COVID-19 outbreak, the epidemiologic criteria for the identification of a suspected case were updated with travel record to Wuhan and direct contact from patients of Wuhan having respiratory symptoms or fever by the WHO on January 18, 2020. The definition for confirmed case was based on the positive result for SARS-CoV-2 detection on the respiratory specimen by virus isolation method or at least two positive test results by real-time reverse-transcription–polymerase chain reaction (RT-PCR) or by determining the viral genome sequence by sequencing[2].

Coronaviruses (CoV) cause different diseases varying from common cold towards severe diseases like Middle East respiratory syndrome (MERS-CoV) and severe acute respiratory syndrome (SARS-CoV), also causing death due to pneumonia, respiratory failure, cardiac arrest, kidney failure, or multi-organ failure. The SARS-CoV-2 is a new coronavirus strain reported in human beings. It is not possible to differentiate COVID-19 caused by SARS-CoV-2 from other respiratory infections of viral, bacterial, mycotic, or mycoplasma etiology. Accordingly, travel history is important; yet, during the community transmission phase, travel history becomes irrelevant[6]. SARS-CoV-2 was preliminarily identified by Koch's postulates and viral morphology, as determined by electron microscopy[7]. Signs of COVID-19 are fever, dry cough, and difficulty in breathing. The spread of virus can be controlled by regular and proper washing of hands , covering of mouth and nose during coughing and sneezing, properly cooking meat and eggs before consuming, and maintaining at least one-meter distance with anyone[8]. This disease is a major threat, as it can be transferred from both symptomatic and asymptomatic carriers, particularly young children (who typically show less pronounced symptoms). Early and accurate detection based on both clinical symptoms and molecular evidence is important for the preliminary, presumptive and confirmatory diagnosis of COVID-19[10,11].

This paper aimed to provide information and guidance to laboratory personnel, stakeholders, and public health professionals involved in laboratory testing of samples collected from patients suspected for COVID-19. Basic information about laboratory tests currently used for COVID-19 is reviewed for public health professionals as well as the community in general. WHO documents and data from the literature, based on searches against the Internet, Google Scholar, and PubMed Central (PMC) with the keywords as laboratory tests for SARS-CoV-2, WHO

guidelines for COVID-19, pandemic, RT-PCR, and COVID-19 virus are summarized.

Diagnostic testing for COVID-19

COVID-19 diagnostic testing is essential for tracking SARS-CoV-2, revealing its epidemiology, sharing data, and preventing transmission[12]. Importantly, to halt the spread of COVID-19, testing is critical. Data on testing is also important because it allows infected individuals to be aware of their infection status, enabling individuals to receive appropriate care and provide a basis for governments to implement measures to reduce the probability of community transmission. Testing plays critical role for follow up of adequate prevention and control strategies to counter the COVID-19 pandemic. It provides insight into the spread of the disease and provides a basis for evidence-based strategies to be implemented for controlling the transmission and further spread of the disease in the community. Rapid collection and testing of appropriate specimens from suspected cases is a priority for clinical management of patients and to control the spread of disease. Testing should be performed under the guidance of a laboratory expert. The suspected cases should be tested for SARS CoV-2 virus genome by nucleic acid amplification tests (NAAT), such as reverse transcription polymerase chain reaction (RT-PCR). All the non-propagative diagnostic procedures on the specimens collected from patients suspected or confirmed for COVID-19 can be conducted in BSL-2 laboratories. The handling of high concentrations of live virus for isolation or propagation or neutralization studies should be performed only at the BSL-3 containment facilities by properly trained, qualified and competent personnel with personal protective equipment[13-14]. Personal protective equipment (PPE) is critical for laboratory diagnostic procedures; however, its effectiveness is highly correlated with staff training, hygienic practices, laboratory facilities, laboratory design, and other human factors[15].

Biosafety Level 2 Laboratory (BSL2)

This BSL2 laboratory is most commonly used for primary health services, diagnostic and research purposes. The laboratory practices include following GMT (good microbiological techniques), use of protective clothing, and display of biohazard signs. The main safety equipment available in BSL2 laboratories is Biological Safety Cabinet for protecting the laboratory personnel handling the clinical specimens and the environment.

Containment Biosafety Level 3 Laboratory (BSL3)

This laboratory is for special diagnostic services such as virus isolation and research involving virus propagation on any pathogen that has potential to spread through aerosol. Laboratory practices include special clothing, restricted access, directional airflow, and highly trained personnel. Safety equipment includes biological safety cabinet, autoclave, and other devices for all activities[16]. Any breach in the laboratory practices may lead to escape of dangerous pathogens. Laboratory personal have to be well trained and all the standard operating procedures (SOP) should be followed.

According to the WHO document on laboratory guidelines, the decision to test needs to be relied on clinical symptoms, epidemiological factors, and associated to an initial assessment of the possibility of infection. It is critical when adequate number of tests are not available. SOPs should be in place for all the procedures and it should be strictly followed. The working staff should be trained for the collection, storage, packaging, and transport of the specimen. Since all bio-samples are considered potentially infectious, persons conversant in the appropriate use of PPE and taking all necessary precautions for sample collection, handling, labeling, processing, storage, and transport should be employed as per standard protocols[17]. The CDC recommends that persons involved in the diagnosis of COVID-19 should be equipped with all necessary PPE, such as laboratory aprons, head caps, face masks (e.g., N95, N100, and PAPR), hand gloves, back-side closure gowns, goggles for eyes or a facial shield, and completely closed footwear and/or disposable shoe covers to ensure a barrier between the testing staff and patients[18,19].

Samples should include clinical bio-samples from the lower or upper respiratory tract of patient viz., bronchoalveolar lavage, oropharyngeal swabs, sputum, nasopharyngeal swabs, endotracheal aspiratory fluid, conjunctival swabs, tears, blood, serum, and feces, depending on the clinical symptoms[7,20-23]. All specimens collected from suspected cases or community for COVID testing should be regarded as potentially infectious and the national guidelines for testing

should be strictly followed in all circumstances. Certain mandatory requirements in terms of the suitability and health status of manpower, laboratory design and infrastructure, precautions and protocols, and biosafety requirements for COVID-19 diagnosis are in place which must be meticulously followed[18]. Handling of the samples / specimens for molecular testing requires BSL-2 facilities, while isolation and culturing of the virus need BSL-3 facilities. Additional samples such as blood and stool can be collected for SARS-CoV-2 detection as coronaviruses have been detected in these samples. The correct handling of specimens during transport is essential and it should reach the laboratory at the earliest feasible time. Collected specimens should be transported at 2–8°C under normal circumstances. If there is a delay in transporting the specimen, the use of viral transport medium is advised and sample can be frozen at –20°C or -70°C and packed with dry ice. The repeated freezing and thawing of specimens shall be avoided[13].

Basic principles of laboratory techniques:

PCR requires DNA extraction by the following methods: organic extraction, silica-based technology, anion exchange technology, and magnetic separation. After excluding poor-quality DNA, PCR is performed for the amplification of a single copy or a few copies of a segment of DNA to obtain millions or billions of copies for virus detection. The procedure was invented in 1983 by Kary Mullis, who won a Nobel Prize in 1993. The PCR generates millions of copies of target DNA or RNA for analyses, visualization by gel electrophoresis, sequencing, or cloning into plasmids to be used for further experiments[24]. PCR is commonly used in the diagnostic laboratories for molecular diagnosis of various pathogens. The PCR works by setting up a reaction in a tube and then subjected to cycle steps using programable thermal cycler.

Reverse transcription PCR

RT-PCR is employed to detect expressed RNA, viral genomic RNA, and other RNAs. Traditionally, it involves a two-step procedure (i.e., reverse transcription and PCR), but one-step RT-PCR using Tth Polymerase has been developed. Reverse transcription is the process by which RNA is converted into a complementary DNA (cDNA) using the enzyme reverse transcriptase, a unique enzyme obtained from retroviruses. Several reverse transcriptases are used for this purpose, such as MMLV, AMV, Superscript-I, Superscript-II, Superscript-III, and others from various suppliers. To detect the genome of RNA viruses, such as SARS-CoV-2, RT-PCR is an important approach.

PCR components

1. **Taq DNA Polymerase:** PCR requires a thermostable DNA polymerase enzyme which can copy and make new strands of DNA using existing strands as templates.
2. **Buffer**: PCR buffer of 10x concentrate needs to be diluted 1:10 in the final PCR. It provides optimal conditions for DNA synthesis by DNA polymerase.
3. **Magnesium Chloride:** The $MgCl_2$ concentration can affect the reaction specificity and efficiency. Polymerases require divalent cations for their activity. Standard PCR buffers contain 1.5 mM $MgCl_2$.
4. **dNTPs:** The dNTP mixture includes four nucleotides (dATP, dTTP, dCTP, and dGTP), which are the building blocks of the new DNA strand. It is necessary to make new copies of DNA.
5. **Primers (Forward and Reverse):** A primer is a short sequence of nucleotides (generally 18–24 nucleotides) that serves as a starting point for DNA synthesis. It is essential because the DNA polymerase such as *Taq* polymerase which synthesize the new strand of DNA can only add new nucleotides at the 3'end of an existing strand of DNA and copies the opposite strand. Two primers (forward and reverse) are used in each PCR that bind to opposite strands of the template DNA, at the target region edges for exponential amplification. The primers bind to the template by complementary base pairing. The primers confer specificity for a PCR assay by binding to the specific target template DNA through complementary base pairing. For example, in PCR assays for SARS-CoV-2 detection, the primers should be specific to the target virus and should not bind to other closely related viruses, such as SARS or MERS viruses or unrelated viruses. Primer selection is a critical step in PCR.

6. **Template DNA:** The template is purified DNA from clinical samples or cDNA prepared by reverse transcription. The concentration and purity of DNA can affect the efficiency of PCR. A high concentration of DNA and impurities can inhibit the reaction.

Real-time PCR

Real-time PCR is commonly employed for the detection of viruses and other pathogens in diagnostic laboratories owing to its high specificity, direct end-point detection, and automation. This approach is most commonly used for COVID-19 diagnosis in laboratories worldwide.

Real-time PCR is used to qualitatively detect or quantitatively measure and determine the presence of specific DNA sequences. In this technique, a PCR is monitored in real time when DNA is amplified and measured simultaneously throughout the reaction process and the amount of amplification is measured using a fluorescence-based detection system. Real-time PCR measures amount of fluorescence at the end of each cycle during PCR. The amount of amplification is directly proportional to the amount of fluorescence generated in each reaction at the end of each cycle. Real-time PCR requires a light source, optics, and fluorescent molecules (probes) to measure amplification at each cycle of the PCR process, rather than at the finishing point of the final cycle like in conventional PCR. Quantitative PCR and real-time PCR refer to the same procedures. As the Real-time PCR is designed to collect data as the reaction proceeds, an accurate quantitation of DNA is possible using controls.

Real-time PCR requires either a fluorescent dye which binds to double-stranded DNA or a fluorescently labeled probe in addition to other PCR components. Of these, fluorescently labeled probe is more preferred as it can be highly specific to the target DNA. A labeled probe is an oligonucleotide of 25 to 30 nucleotides in length that is modified to contain a fluorophore and a quencher. Under normal conditions, the fluorophore and quencher are held together by the probe nucleotide sequence. Due to their close proximity, the light emitted from the fluorophore is absorbed by the quencher and the net fluorescence is zero. The probe is designed to bind to the target DNA between the forward and reverse primers. During PCR amplification, the nucleotide sequence including the fluorophore and quencher is degraded by the exonuclease activity of Taq polymerase and the fluorophore is released from the quencher. Then, the free fluorophore emits light at a specific wavelength when excited by a light source, which is detected by the optics in the real time PCR instrument. The fluorescent light emitted is directly proportional to the amount of DNA amplified at each cycle. The probe along with the primers increases the specificity of the quantified PCR product.

PCR Cycles

After adding all components of the PCR, appropriate cycling conditions using a thermal cycler are needed for the exponential amplification of target DNA. The main steps in PCR are given below. Steps 1 and 5 are performed once and steps 2 to 4 are repeated 30–40 times.

1. **Initial Denaturation:** This step is performed at 94-95°C for 3 to 5 minutes to convert double-stranded DNA into single-stranded DNA. The primers can only bind to single-stranded DNA. It is carried out only once while starting the reaction.
2. **Cycle Denaturation:** The denaturation step is performed at 94-95°C for 30 seconds - 1 minute at each cycle. The double-stranded DNA is denatured into two single-stranded DNA molecules during this process. The single-stranded DNA is the site of primer annealing during subsequent amplification.
3. **Annealing:** Annealing is performed generally at 55-65°C for 30 seconds to 1 minute at each cycle for allowing the primers binding to target template DNA. The sequence-specific forward and reverse primer binds to single-stranded DNA. Fluorescent dye or probe also binds to DNA sequence in real time PCR during the annealing step.
4. **Extension:** Extension is performed at 72°C for 1-2 minute depending on length of amplified product. Taq DNA polymerase activity is highest during at this temperature and adds dNTPs to the growing DNA strand at this stage.
5. **Final extension:** This step is performed at 72°C for 5 to 10 minutes for completion of the amplifications.

As mentioned above, cycling steps 2–4 are repeated 30–40 times to generate enough copies of DNA[25].

The amplification plot contains quantitative information about DNA amplification. At cycle threshold (Ct) value, fluorescence is measurable. Baseline refers to the initial timepoint at which fluorescence is nearly zero. At exponential phase, amplification reaches a peak[25]. Absolute quantitation of virus copies can be performed using appropriate known copy number controls. Relative quantitation is also possible by determining the copy number relative to those of housekeeping genes, such as beta-actin, beta-globin, or *GAPDH,* which are constitutively expressed in host cells.

Real-time PCR is widely used for the detection of SARS-CoV-2. Laboratory diagnosis of SARS-CoV-2 was confirmed for first time by the Chinese Center for Disease Prevention and Control (CDC) and then, the National Health Commission approved diagnostic confirmation in certified tertiary hospitals across all provinces of China. RT-PCR assays were carried out as per protocol defined by the WHO. Nucleic acids extraction from respiratory samples was carried out using commercially available kits. The China CDC employed the following primer sequences for SARS-CoV-2 for RT-PCR assays. For open reading frame 1 ab fragment, primer sequences were: forward, 5′-CCCTGTGGGTTTTACACTTAA-3′, reverse 5′-ACGATTGTGCATCAGCTGA-3′, probe 5′-FAM-CCGTCTGCGGTATGTGGAAAGGTTATGG-BHQ1-3′. For the N region of the viral sequence, the primer sequences are as follows: forward 5′-GGGGAACTTCTCCTGCTAGAAT-3′, reverse 5′-CAGACATTTTGCTCTCAAGCTG-3′, probe 5′-FAM-TTGCTGCTGCTTGACAGATT-TAMRA-3′. They followed initial amplification at 50°C for 10 min and 95°C for 5 min, 40 cycles of 95°C for 10 s and 55°C for 40 s. A Ct value of 40 or greater denoted negative findings, whereas a Ct value of less than 37 indicated SARS-CoV-2 detection. A Ct value of greater than 37 but lower than 40 indicated a need for retesting. If two targets (open reading frame 1a or 1b, nucleocapsid protein) were found positive by specific real-time RT-PCR, the case is identified as laboratory-confirmed. Negative results should not be neglected and these must be combined with other clinical observations, patient history, and epidemiological information[26].

First reported patient in the USA was diagnosed by the US Centers for Disease Control and Prevention on January 20, 2020. The viral sequence, replication characteristics, and cell culture tropism were recognized. The virus replicated to high titers in Vero-CCL81 cells and Vero E6 cells in lack of trypsin. The virus was deposited to two virus repositories and made widely accessible to the public health and research communities to speed up developing effective diagnostics, therapeutics and vaccines[27]. The virus genome was sequenced and the sequence was submitted to the NCBI database under the name severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/INMI1/human/2020/ITA (Accession No: MT066156.1)[28].

Several assays for SARS-CoV-2 detection have been developed or are being developed Some assays may detect only the novel virus and few may also detect other strains (e.g., SARS-CoV) sharing high sequence similarity. Some groups have shared their protocols[17,13]. There are automated systems being used for COVID-19 virus detection. Abbott's molecular test for SARS-CoV-2 that run on the Abbott m2000 Real Time System using has been used in USA[29]. Similarly, Cobas systems are one of those supplied by the Roche. According to Roche, there are about 695 cobas 6800 Systems worldwide and 132 cobas 8800s, including more than 100 in the USA. The cobas SARS-CoV-2 Test, a real-time RT-PCR test, is a single-well dual-target assay for specific detection of SARS-CoV-2 and pan-*Sarbecovirus* (subgenus that includes SARS-CoV-2) detection. It utilizes the cobas 6800/8800 Systems with a full-process negative, positive, and internal controls. The cobas 6800/8800 systems are based on a fully integrated and fully automated modular design[30].

The Ministry of Health, Govt. of India and the Indian Council of Medical Research, Govt of India (ICMR) are working closely to address this pandemic with the utmost urgency and to involve both the government as well as private laboratories. ICMR has taken steps for development and training across India. ICMR and KMSCL (Kerala Medical Services Corporation Ltd.) have chosen the Bio-Rad CFX96 Touch Real-Time PCR systems for laboratory testing in India[31]. In India, Mylab, Pune-based molecular diagnostic company, is the first Indian company to receive

approval for its RT-PCR based Covid-19 diagnostic test kits from the Drug Controller General of India, after the validation from the ICMR and the National Institute of Virology. The Mylab research team claims that their kit yields a diagnosis in 2.5 hours, while imported kits take 6–7 hours. Their kit is called Patho Detect and was developed within a short period of 6 weeks instead of the typical timeframe of 3–4 months[32].

RT-PCR has a major limitation with respect to biosafety, including the processing, handling of bulk biological samples of patients who are potentially infectious, the long test duration, and laborious procedure involving several critical steps[33]. CT scans are an essential auxiliary diagnostic tool used by some clinicians to circumvent the false-negative results obtained by RT-PCR[34-35].

A variety of other molecular diagnostics tests, like recombinase polymerase amplification, multiplex nucleic acid amplification, helicase-dependent amplification, loop-mediated isothermal amplification (LAMP), reverse transcription LAMP (RT-LAMP)[36] isothermal loop-mediated amplification of coronavirus (i-LACO), next-generation sequencing (NGS), and microarray-based assays have also been employed in several labs for the molecular confirmation of SARS-CoV-2 infection[37,38]. Other diagnostic tools targeting SARS-CoV-2 nucleic acids and/or proteins for the diagnosis of COVID-19 include CRISPR-based SHERLOCK multiplexed signal detection via fluorescence, biomarker CRISPR/Ca9-mediated lateral flow nucleic assay (CASLFA), reverse polymerase amplification (RPA), rolling circle amplification (RCA), real-time nucleic acid sequence-based amplification (NASBA), quantum dot barcodes employing multiplexed quantum beads, enzyme-linked immunosorbent assay (ELISA)[39] ELISA-based smartphone dongle for quick detection, digital ELISA-based single molecule array (SIMOA), DNA-assisted immunoassays, like bio-barcode assay, and magnetic separation of target proteins by paramagnetic beads using a magnetic biosensor[40-41].

Serological markers are also being evaluated; however, they are not yet sufficiently effective for confirmatory diagnosis[42]. Moreover, cross-reactions with other coronaviruses could affect the outcome of serological diagnosis[43]. Yet, several serological tests providing rapid results may be useful for community surveillance[44,45]. The COVID-19 Rapid Test applies lateral flow immuno-chromatography to qualitatively detect IgG and IgM antibodies to SARS-CoV-2; this IgM-IgG combined assay is ease in use and has higher sensitivityas compared to a single IgM or IgG test and can rapidly screen both symptomatic and asymptomatic carriers[46]. Point-of-care testing for detecting the type and titer of IgM/IgG based on immune identification technology is also helpful for serological detection. Serological confirmation is obtained in paired serum samples when the antibody titer is four-fold higher in convalescent serum than in acute phase serum from the same patient. Rapid research progress related to COVID-19 has made it mandatory to keep abreast with the updated guidelines of individual countries for the diagnosis of suspected and confirmed cases[47].

Nodal centers of the Viral Research and Diagnostic Laboratories (VRDL) are designated testing sites. Directives were developed for use by government health authorities and organizations, hospitals, clinicians / doctors, and for planning of laboratories for collecting suitable clinical samples for the diagnosis of COVID-19. ICMR issues guideline and policies for the collection, packaging, and transport of clinical samples / specimens to the ICMR-National Institute of Virology (NIV), Pune, Maharashtra for COVID-19 diagnosis. Clinicians are responsible for determining the necessity for specimen collection for laboratory testing for SARS-CoV-2 after adopting the case definition provided by health authorities. Appropriate clinical samples are required to be collected by trained laboratory staff or healthcare workers under guidance of a clinician while following necessary biosafety precautions, using PPE and the clinical samples should be sent to the designated laboratory following standard triple packaging[48]. ICMR recognized various viral research and diagnostic laboratories at the regional level across 28 states and union territories for COVID -19 testing. As of May 11, 2020, a total of 16,73,688 samples have been tested for SARS-CoV-2[31], with 44,029 confirmed active cases, 20,916 cured cases, and 2,206 reported deaths in India [31].

CONCLUSION AND FUTURE PROSPECTS

In addition to strengthening laboratories, it is important to constitute an effective and active system that can design safe, protective vaccines, immunotherapeutics and antiviral drugs. This goal presents technical, diplomatic, and budgetary challenges and benefits from partnerships between the public and private sectors. The development of antiviral drugs and vaccines require various clinical trials, needful approvals and licensing agreements to reach to bulk production phases by pharmaceutical companies and manufacturers. Relevant global platforms include the WHO R&D Blueprint, the Global Research Collaboration for Infectious Disease Preparedness and the International Severe Acute Respiratory and Emerging Infection Consortium trial network[49]. Medicines, PPE, sanitizers, and testing facilities should be available and affordable in outbreak areas and areas in greatest need. This strategy is ethical and is also essential for ending the community transmission chain and preventing future pandemics. The further expansion of testing capability, investigations, surveillance, strengthening of infrastructure, upgrading technologies, appropriate funding, sufficient supply of logistics, developing an informed and adequate health workforce, and decision-making to preclude the worst outcomes are essential. Governments should focus on rejuvenating the health sector, especially public health preparedness, in the future to prevent outbreaks of this enormous scale.

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CONFLICT OF INTEREST

The authors declares that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All the listed author(s) have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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AVAILABILITY OF DATA

Not applicable.

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