A Review Article on COVID-19

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Abstract: The aim of this study was to give a brief explanation about COVID19, novel coronavirus that is a global pandemic right now, its causes, factors that can cause COVID19, its origin, its recent effect, diagnosis, treatment etc. in India and all over the world. Also given a brief description of coronavirus and its types. COVID19 is causing many deaths at an alarming rate all over the world right now. Worldwide it is causing complete lockdown and given safety measures are must in order to prevent it from spreading further. According to recent estimation worldwide 69,31,000 cases are reported taking lives over 4,00,857 people globally. In India the number of cases is approximately 2,56,611 with number of deaths reported 7,135. Globally, as of 4:00pm CEST, 8 June 2020, there have been 6,931,000 confirmed cases of COVID-19, including 400,857 deaths, reported to WHO and these numbers are continuously increasing day by day. It has been declared as global pandemic by WHO. It can occur in all age groups but older people and patients with previous disease conditions are at higher risks. Researchers all over the world are continuously looking for cure or vaccine; clinical trials are done for expected agents as soon as possible to slow down this king of virus. Though we cannot completely exclude COVID19 from world but if its cure or vaccine is discovered then its effects can be minimized. Due to unavailability of vaccine or medicinal cure for the virus it is causing devastation even having lower mortality rate than other types of corona virus. COVID 19 has a mortality rate of 5.5% which changes according to age and area as it is highly contagious and can be transmitted from human to human. It is continuously mutating also so it’s really important to find a warrior for COVID19.

Keywords: Coronavirus, COVID19, pandemic, mortality rate, vaccine.

INTRODUCTION
Coronavirus typically affects the upper respiratory tracts of birds and mammals, including humans. Researchers associate them with the common cold, bronchitis, pneumonia, severe acute respiratory syndrome (SARS) and coronavirus disease of 2019 (COVID19). These can also affect the gut. Over the past 80 years, scientists have found that these viruses can infect mice, rats, cats, dogs, turkeys, horses, pigs and cattle. Sometimes these animals transmit the virus to humans also. Most recently authorities identified a new coronavirus outbreak in China that has no reached other countries also. The virus was named to be SARS-COV-2 that causes COVID19. Researchers first identified a coronavirus in 1937, one that was responsible for a type of bronchitis in birds. Scientists found evidence of human coronavirus in 1960s, in the noses of people with common cold. Human corona viruses that are particularly prevalent include 229E, NL63, OC43 and HKU1. The name of coronavirus comes from its crown like projection on their surface. Corona in Latin means crown.

WHAT IS COVID19
In 2019, the Centers of Disease Control and Prevention (CDC) started monitoring the outbreak of a new coronavirus, SARS-CoV-2 which causes COVID19. Authorities first identified the virus in Wuhan, China. And due to its spread in nearly every country it was declared a global pandemic by WHO. The first patients with COVID19 were found to be linked by an animal and seafood market which suggests this virus was initially transmitted from animals or birds to humans. When cases of patients not connected with the seafood market were reported, it confirmed the human to human transmission of the virus. Corona virus is common in people and many different animals including cats and bats. Commonly, human corona virus typically causes upper respiratory infections like common cold. Most people get infected by one or more type of these viruses at some point in their life. This infection resolves on its own with basic rest while feeling miserable. Rarely the coronavirus that infects animals can evolve and become human coronavirus which infects and spread through person to person. Basic types of human corona virus are severe acute respiratory syndrome-SARS (2003) and Middle East respiratory syndrome- MERS (2012).

**TIMELINE FOR COVID19**

In December 2019, Wuhan a city in China noticed unusual increase in number of cases with pneumonia in hospitals. These patients mostly showed the common clinical symptoms of dry cough, fever, dyspnoea and bilateral lung infiltration imaging. The corona virus cases were eventually reported to WHO office in China.

On December 31, 2019 many researches took place to search the source of coronavirus and the origin was found to be the sea food market and wet market in Wuhan. The market included large variety of vertebrates and invertebrate animals and it was estimated that it was spread from bats to humans.

On January 1st, 2020, the said market was closed and surrounding area was quarantined.

On January 7th, 2020, Chinese scientists identified the pathogen responsible for pneumonia as the novel corona virus.

On January 11th, 2020, China recorded their first death by the novel coronavirus and a day after shared the genetic sequence of the novel coronavirus with rest of the world which was important in diagnostic and research practices for the novel coronavirus.

On January 13th, 2020, first case of that virus was reported outside China in Thailand.

On January 20th, 2020, first US recorded their first case for the said virus.

This was followed by first 3 cases of novel coronavirus in Europe on 24th January 2020.

By the end of January 2020, the source of coronavirus was found to be wild bats and belong to similar group as SARS; hence this one was known as SARS-CoV-2.

On January 30th, 2020, WHO declared SARS-CoV-2 as a global public health emergency (outbreak). And India also reported their first case for this corona virus.

On 2nd February, 2020, the first death by SARSCOV2 was reported outside China in Philippines.

On 11th February, 2020, WHO announced the official name as COVID19 as a short version of coronavirus disease of 2019 and the cause was SARSCOV2.

On 15th February, the first death in Europe was reported in France.

On 19th February the COVID19 outbreak occurred in Iran.

On 11th March, COVID19 was declared as a global pandemic. It is the first pandemic caused by coronavirus.

On 20th March, Italy surpassed China as a country with highest death toll.

On 24th March, Japan’s PM and International Olympics Committee agreed to postpone the Olympics until 2021 amid the outbreak.

On 26th March, the number of cases globally exceeded 500,000.

On 29th March, the US has reported highest number of cases globally followed by Italy and Spain.

On 2nd April, number of cases worldwide surpassed 1 Million and the cases continued to increase day by day.

**COMPARISON WITH OTHER HUMAN VIRUSES**

COVID19 is highly transmissible. The Reproductive Number, that is the number one infected person will infect on average which also suggest how infectious the disease is was found to be 2.2 higher the Reproductive Number, more infectious is the disease. If we compare COVID19 with common cold caused by influenza the reproductive number is about 3. For smallpox, it is about 6 and for measles it is about 9-18. The number of COVID19 patients is increasing at a faster rate is because it is contagious and due to availability of diagnostic kits and awareness also. As of April 6th, it infected over 13,00,000 people and killed over 70,000 people worldwide and carried a mortality rate of 5.5% which varied between countries. In Italy the mortality rate reached up to 10%.

If we compare COVID19 with other types of corona viruses, SARS (2003) had over 8000 cases with a mortality rate of 35% and MERS (2013) had over 2500 cases with the mortality rate of 35%.

Despite having low mortality rate than others, 5.5% is still very significant specially with rapidly growing number of cases. As for 100 infected people, 5 patients may die. For influenza which affects millions, the mortality rate is less than 0.2%.

- The following graph represents the number of cases worldwide till 8th June, 2020.
CASE COMPARISON DATED TILL JUNE 9, 2020

WHO REGIONS

AMERICAS
3,311,387 confirmed cases

EUROPE
2,286,560 confirmed cases

EASTERN MEDITERRANEAN
641,429 confirmed cases

SOUTH-EAST ASIA
364,196 confirmed cases

WESTERN PACIFIC
191,275 confirmed cases

AFRICA
135,412 confirmed cases

PHARMACOLOGY OF COVID19

COVID19 is caused by SARSCOV2, a beta coronavirus. It is composed of single stranded (ss) RNA protected by lipid bilayer and membrane protein which also contain surface proteins called “spikes”. The virus targets and infects respiratory system and is transmitted by contact, droplets and fomites from an infected person who can be either symptomatic or asymptomatic. The incubation period which is the time of infection to the appearance of symptoms is about 2-14 days. During this the virus slowly triggers the air response within the lungs. The lungs contain sacs of alveoli where gaseous exchange takes place, it contains alveolar cells which produce surfactants to coat inner lining alveoli to keep it open for gaseous exchange. SARSCOV2 mainly invades alveolar epithelial cells which resulting respiratory symptoms. It targets and binds to ACE II which is a receptor as well as enzyme on type2 alveolar cells where the surface proteins (spikes) binds. ACE II is required to gain entry inside the cells and this suggest
that SARS-CoV-2 has similar pathogenicity as SARS. Coronavirus generally enters via endocytosis by direct fusion of viral envelope with the host membrane. Once inside virus particle is uncoated and its genome enters the cell’s cytoplasm. Since coronavirus has single positive RNA genome, they can directly produce their proteins and new genome in cytoplasm by attaching to the host ribosomes. It will translate the viral RNA to form proteins that will make RNA polymerase which will read the positive strand to make a negative RNA strand. This strand will then be used to make positive RNA strand and other small RNAs. Small RNAs will be read by host ribosomes in their endoplasmic reticulum to help them make structure components of virus. These will be sent to golgi apparatus where these will be nicely packaged with positive RNA to form new viruses. These progeny viruses are then expelled out of the cell by exocytosis through secretory vesicles. While the virus is self replicating in the alveolar cells it also damages it and this will initiate the inflammatory response.

Injured alveolar cells release interferons, cytokinins as well as intracellular components. Interferon acts in a paracrine manner and has numerous effects on surrounding cells preparing them to war off viral infections. The primary function is to induce protection in neighboring non infected cells against viruses. Alveolar macrophages detect cell injury via damage associated molecular patterns from the alveolar cells. They also respond to cytokines released I injured alveolar cells; this causes macrophages themselves to secrete cytokines such as TNFα, Int-1, Int-6, Int-8 and other chemokines. The inflammatory response occurring in parenchyma stimulates the nerve ending responsible for initiating cough reflexes. TNFα and Int-1B are proinflammatory cytokines that cause increase in vascular permeability and increase in adhesion of molecules. This allows recruitment of other immune cells including neutrophils and monocytes. They will bind to these adhesion proteins and enter the site of injury. Int-8 will recruit neutrophils and other chemokines will will attract monocytes. This increase in permeability induces leakage of fluids into the interstitium causing oedema and into the alveoli causing pulmonary oedema. This will cause dyspnoea and impaired oxygenation leading to hypoxaemia. The increase in circulating macrophages and neutrophils lead to increase in WBC count in serum while neutrophils are important in acute setting by engulfing viruses and debris around the area, it can also be detrimental after a while because it releases chemicals as a byproduct damaging the surrounding tissue. There will be damaged alveolar cells all over that means less surfactant being produced and hence th alveoli will easily collapse resulting in impaired oxygenation and hypoxaemia. The WBC and other damaged endothelial cells release other inflammatory mediators, the arachidonic metabolites including leukotrienes and prostaglandins. Leukotrienes will cause bronchoconstriction impairing ventilation and hypoxaemia. PGs, Int-6 and TNFα all are responsible of causing fever, a prominent feature of COVID19. Decrease oxygen level in blood will stimulate chemoreceptors in the aortic arch and in the brain. Decrease will stimulate cardiopulmonary centers in the brain to tell the lungs to breathe more to increase oxygen level in blood and also tell heart to pump faster to deliver oxygen to the body and that’s why patients with hypoxaemia are tachypnoea and tachycardiac.

Some people develop asymptomatic COVID19 as their immune system keeps it in check or they develop only minor symptoms such as mild cough and shortness of breath will small fever. The alveolar macrophages can detect the virus using its special receptors called Tolac receptors (TLR). It engulfs virus particles through phagocytosis, process it and then show it on the surface. Studies have shown that the spike protein of the virus could be presented and due to this specific T-cell may recognize and mount to immune response consisting of b-cells or plasma cells which will then produce antibodies against the viral spike proteins. In viral infections a common finding is lymphopaenia due to release of interferons for infection. Int-6 is a potent proinflammatory cytokines which has effects like stimulating hepatocytes to release acute phase reactants including CRP, fibrinogen and hepcioin. CRP measured in blood is a good marker of inflammation.

**ACUTE RESPIRATORY DISTRESS SYNDROME**

In summary the injured lung, accumulated fluid, ventilation perfusion mismatch and hypoxaemia that is not related to heart function is what is called ARDS. And is leading cause of mortality in patients with COVID19. And ventilators are used to let patients with severe condition breathe. It provides the optimal oxygen level needed and also give them the pressure they need in order to keep the lung sacs open. **Ventilators** are used to let the patient with severe shortness of breathing.
When oxygen level is really low these devices are used to give them optimal oxygen supply in bloodstream. It also give them the pressure they need in order to help keep the lung sacs open.

STAGES
People get varied degree of severity with COVID19.
80% of majority cases will get mild infections.15% get severe disease with dyspnea, hypoxia and lung changes on imaging. While 5% are actually critically ill with respiratory failure with ARDS, shock or multiorgan dysfunction including liver derangement and heart can release troponin, which is a marker of cardiac injury. Some severe patients also have laboratory evidence of juvenile inflammatory response similar to cytokine release syndrome.

CLINICAL MANIFESTATION PRESENTATION AND RISK FACTOR
Median age range is 49-56 years and surprisingly children are typically asymptomatic or present with mild symptoms. Males and females are equally affected.

SYMPTOMS
- Fatigue
- Dry cough
- Anorexia
- Myalgia
- Dyspnoea
- ARDS require intensive care

The global mortality rate is about 5.5% that increases with age and co morbidities like diabetes, heart disease, chronic kidney disease, chronic lung disease and the situation is evolving rapidly.

DIAGNOSIS
- Investigations finding of people infected with SARSCOV2 are lymphopaenia and if one has pneumonia or severe cases most of them will develop leukocytosis, elevated white cell count, elevated liver transaminases, elevated lactic dehydrogenases and elevated CRPs.
- Chest X-ray is important in all cases of pneumonia. You can get unilateral or bilateral lung infiltrate in severe cases.
- A CT of chest in patient most commonly demonstrates ground glass capacity with or without consolidation.

RTPCR: REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION
To perform this test the sample is taken from nasopharyngeal swab or oropharyngeal swab. For nasopharyngeal specimen the swab is inserted in nostril and gently moved forward into nasopharynx. Then it is rotated for a specified period of time to collect secretion containing virus. The swab is removed gently and is immediately placed into sterile tube containing a viral transport medium. The standard method of corona virus testing is PCR which is a method widely used in molecular biology to make millions and billions copies of specified DNA fragment rapidly. Corona viruses contain an extraordinary long ss RNA genome. To detect these viruses through PCR, RNA molecules must be converted into their complementary DNA sequence by reverse transcriptase, then the newly synthesized DNA can be amplified by standard PCR procedure. This approach is universally known as RTPCR. To perform this technique basically viral DNA has to be extracted. Several RNA purification kits are available for convenient, fast and effective isolation.
1. **Extraction:**
   To extract the viral RNA by using commercial kit sample is first added into microcentrifuge tube then it is mixed with **lysis buffer**. This buffer is highly denaturing and usually consist of phenol and Guanidine isothiocyanate. RNAase inhibitor are also usually present in lysis buffer to ensure isolation of intact viral RNA.

2. **Denaturation:**
   Once the lysis buffer is added the tube is mixed by pulse vortexing and incubated in room temperature. Then the virus is lysed at highly denaturing conditions provided by the lysis buffer.

3. **Purification:**
   Once the sample is lysed the purification procedure is carried out by using a spin column. The sample is loaded onto the spin column and centrifugation is performed. This procedure is a solid phase extraction method in which the stationary phase is of silica matrix. Under optimal salt and pH conditions the RNA molecules are bind to the silica gel membrane and at the same time protein and other contaminants are not retained.

4. **Washing:**
   After centrifugation spin column is placed into a clean collection tube and the filtrate is discarded. Then a wash buffer is added. The column is put in the centrifuge again forcing the wash buffer through the membrane. This removes any remained impurity from the membrane leaving only RNA bound to silica gel.

5. **Extraction by elution:**
   Once the sample is washed the column is placed in a clean centrifuge tube and an elution buffer is added and centrifugation is carried out forcing the elution buffer through the membrane. The elution buffer removes viral RNA from the spin column and a purified RNA which is free of protein, inhibitors and other contaminants is obtained.

6. **Preparation of master mix:**
   After the extraction of the viral RNA the next step is preparation of reaction mixture for PCR amplification. In this step a master mix is used which is premixed concentrated solution that consists of buffer, reverse transcriptase enzyme, nucleotides, forward and reverse primer, **TaqMan** probe and DNA polymerase. Finally to complete this reaction mixture the RNA template is added.

7. **PCR:**
   The tube is mixed by pulse vortexing. Then the reaction mixture is loaded into PCR plate which generally contains 96 wells allowing the analysis of several samples at the same time. Next the plate is placed into the PCR machine which is essentially a thermal cycler. Real time RTPCR is used for the detection of novel corona virus 2019 by the amplification of target sequence in RdRP gene, E gene and the N gene.
The choice of the target gene depends upon the primer and probe gene sequence. The first step in RT-PCR is reverse transcription. The first strand of complementary DNA synthesis is primed with PCR reverse primer which hybridizes to a complementary strand of viral RNA genome. Reverse transcriptase then adds DNA molecules onto the 3’ end of the primer synthesizing DNA complementing the viral RNA. The temperature and duration of this step depends on the primer, the target RNA and the reverse transcriptase used. Next an initial denaturation step is applied initiating denaturation of DNA-RNA hybrid. This step is required for activation of DNA polymerase and simultaneously for the inactivation of reverse transcriptase. PCR consists of various steps of thermal cycles where each cycle consists of denaturation, annealing and extension.

Denaturation includes heating the reaction chamber up to 95°C and it is used for denaturation of ds DNA template. Annealing includes reaction temperature to be lowered till 58°C allowing the annealing of forward primer to its complementary part of ss DNA template.

Extension includes the DNA polymerase synthesizing a new DNA strand complementary to DNA template strand by adding free nucleotide. Reaction mixture that is complementary to template in the 5’→3’ direction. The temperature in this step depends upon the DNA polymerase used.

After the first cycle the ds DNA target is obtained then the denaturation of this ds DNA is performed yielding two ss DNA molecules. In the next step reaction temperature is lowered allowing the annealing of primers to each of the strand of DNA template and annealing of TaqMan probe to its complementary part of target DNA. TaqMan probe consists of flora for covalently attached to the 5’ end of the oligonucleotide probe. The fluorescence is emitted by the flora when it is excited by the cycler’s light source. Also, this probe consists of quencher at 3’ end. The close proximity of the reporter to the quencher prevents detection of its fluorescence. During extension DNA polymerase synthesize new complementary strands. When the polymer reaches TaqMan probe its endogenous 5’ nuclease activity cleaves probe separating dye from quencher. With each cycle of PCR, more dye molecules are released resulting in increase in fluorescence intensity proportional to the amount of applicant synthesized. This method allows the estimation of amount of given sequence present in a sample. The number of ds DNA strands is doubled in each cycle therefore PCR can be used to analyze extremely small amount of sample.

For the measurement of fluorescence signal a Tungsten-Halogen lamp, an excitation filter, mirror, lens, emission filters and a charged couple device (CCD) camera is used. Filtered light from lamp is reflected on mirror through a condensing lens and is focused into the center of each well. Then the fluorescence light emitted reflects from mirror to emission filter and detected by the CCD camera and then converted into digital data.

**MATERIALS AND METHODS**

**Clinical specimens**
- A total of 135 clinical specimens were collected from a cluster of patients with pneumonia in Wuhan and Beijing suspected of being infected with SARS-CoV-2. Specimens included alveolar lavage, sputum, throat swabs, and stool. Inactivation of specimen processing was performed in a biosafety level 3 (BSL3) laboratory.

**Nucleic acid extraction**
- Nucleic acids were extracted from a 140 μl processed specimen using a QIAamp Viral RNA Mini Kit according to the manufacturer’s instructions. Approximately 60 μl of total nucleic acid eluate was recovered into nuclease-free tubes and either tested immediately or stored at -70 °C.

**Design of primers and probes**
- Multiple primer and probe sets were designed based on bioinformatics analysis of three complete genomes of SARS-CoV-2 (BetaCoV/Wuhan/IVDC-HB-01/2019, Accession ID: EPI_ISL_402119, BetaCoV/Wuhan/IVDC-HB-04/2020, Accession ID:
EPI_ISL_402120) obtained in our lab (5,8). ORF1ab, E gene and N gene sequences were selected as targets using Primer Premier software version 5 (Applied Biosystems) with the following default settings: primer melting temperature (TM) set at 60 °C; probe TM set at 10 °C greater than the primers at approximately 70 °C; and no guanidine residues permitted at the 5’ probe termini (Figure 1A, Table 1). Previous reported prime and probe sets for the RNA-dependent RNA polymerase (RdRp) region of pan beta-CoV was designed as a reference experiment (3,9).

Real-time RT-PCR assay for screening of SARS-COV-2 infection

- Several one step rRT-PCR assays were developed using the OneStep PrimeScript™ RT-PCR kit (TaKaRa, Japan). Each 25 µL reaction mixture contained 12.5 µL of 2x Master Mix, 0.5 µL of reverse transcriptase/Taq DNA polymerase mixture, 5 µL of RNA extract, 400 nmol/L concentrations of forward primer and reverse primer, and 200 nmol/L of probe. Thermal cycling included 42 °C for 5 minutes, followed by 95 °C for 10 seconds and then 40 cycles of 95 °C for 10 s and 60 °C for 45 s. Each run included one SARS-CoV-2 genomic template control and at least two negative or mock controls (for the extraction and the PCR amplification step) (Table 1).

Assay specificity was determined using high-titer virus stock as well as clinical samples containing known respiratory viruses from collection of our laboratory. Identities and virus RNA concentrations were reconfirmed by specific rRT-PCRs for each virus before the experiment (9-10).

The limit of detection was independently assessed using a SARS-CoV-2 stock with pre-determined genomic copies. The calibration curve for the genomic copy number versus Ct value was obtained from the rRT-PCR machine, which was an ABI 7500 (Applied Biosystems, USA) or a Roche LightCycler 480 (Roche, Switzerland) Real-Time PCR system. Series of four parallel reactions per concentration step were prepared and tested by the respective rRT-PCR.

Clinical samples were considered positive if two or more of the gene targets showed positive results (Ct≤37 cycles; If the Ct value is between 37-40, the experiment needs to be repeated. The judgment of the final result is based on two consistent experimental results) (7).

RESULTS

Performance of different rRT-PCR methods

- In the detection of clinical samples using the same gradient dilution, ten-fold serial dilutions of the SARS-CoV-2 RNA were tested to assess the detection limits and dynamic range of our optimized rRT-PCR assays. The detection results of the four
rRT-PCR assays were different for clinical samples with gradient dilution. The lower potential limit of detection was approximately 10^4 dilution per reaction for ORF1ab assay, E assay, and N assay, and 10^3 dilution per reaction for RdRp assay (Figure 1C). The genomic copy numbers-based Ct values were calibrated on the standard curve for individual rRT-PCR (Figure 1D). The lowest detection limit of ORF1ab assay was 203 copies/mL. The minimum detection limits of E and N assay were 664 and 667 copies/mL, respectively (Table 1).

The rRT-PCR assays were tested with nucleic acid extracts of 6 human coronaviruses, human NL63 coronaviruses (NL63-CoV), human 229E coronaviruses (229E-CoV), human OC43 coronaviruses (OC43-CoV), human HKU1 coronaviruses (HKU1-CoV), SARS-CoV, and MERS-CoV. In addition, nucleic acid extracts of influenza A were also tested. No positive reactions were observed with any of the primer and probe sets of ORF1ab and N genes, while cross-reactions with SARS-CoV were shown using the primer and probe sets of the E gene as well as RdRp (Figure 1B).

Assay reproducibility was tested by using replicate ten-fold serial dilutions of the SARS-CoV-2 RNA and intra- and inter-assay variability evaluated for each dilution point in quadruplicate three different times. The reproducibility for 3 assays (targeting ORF1ab, E, and N) exceeded 90% at the lower copy detection limit (data not shown).

**Evaluation with different species of clinical specimens**

- The rRT-PCR assay was evaluated using different species of clinical specimens collected from a cluster of patients in Wuhan and Beijing, whom were suspected of being infected with SARS-CoV-2. Three rRT-PCR targeting ORF1ab, E, and N showed consistent detection rates among various species of specimens (Table 2). The mean Ct value detected in sputum was lower than in other species of clinical specimens (alveolar lavage, throat swab, and tool), which indicated the mean viral loads detected in sputum were higher than other specimens. Similar results were also reported recently using a large amount of clinical specimens.

**TREATMENT**

**DISCUSSION**

- To date, several corona viruses (including SARS-CoV, MERS-CoV, and SARS-CoV-2) that can infect humans have all been beta-corona viruses (L,8). The risk associated with false-positive PCR results posed a challenge in clinical application. First, the primer dimers and non-specific amplification, in which probe sequences participate, might interfere with experiments. Second, SARS-CoV-2 assays might cross-react with other viruses. In this study, we used 6 human corona viruses (NL63-CoV, 229E-CoV, OC43-CoV, HKU1-CoV, SARS-CoV and MERS-CoV) and influenza A to test the cross-reactivity. Consequently, the ORF1ab and N gene-based assay were the most specific, exactly matching target genes of SARS-CoV-2. The E gene was cross-reacted with B lineages of the beta-corona virus (such as SARS-CoV). We propose that the E gene could be used as a broad-spectrum screening gene for B lineages of the beta-corona virus, such as SARS-CoV, SARS-CoV-2, bat SARS-like corona virus, et al. Therefore, we currently prefer to recommend the ORF1ab and N gene-based rRT-PCR assays for detection of COVID-19 infection.

- Here, we also verified the sensitivities and detection limits of various rRT-PCR assays. The data showed that similar detection rates and sensitivity were observed among three rRT-PCR assays for ORF1ab, E gene, and N gene targets, which were higher than the RdRp target. We were also noticed that the ORF1ab-based rRT-PCR assay showed the best sensitivity (lowest detection limit).

- To determine which type of specimen is suitable for rRT-PCR in clinic, four species of clinical specimens (alveolar lavage, sputum, throat swab, and tool) were collected for evaluation. Our data indicated that the mean viral loads detected in sputum were higher than other specimens, which suggests that sputum should be a priority for collection for laboratory detection of COVID-19 (11,12).

- Although an rRT-PCR offers clear advantages over more conventional RT-PCR formats, assay results must still be interpreted with caution. For example, the effectiveness of rRT-PCR for detection of SARS-CoV-2 in clinical specimens had been shown to be greatly influenced by the quantity, type, and timing of specimen collection (11,13). In addition, false-negative results due to poor quality nucleic acids or presence of rRT-PCR inhibitors could also be a concern. False-negative results could also potentially arise from mutations occurring in the primer and probe target regions of SARS-CoV-2 genome. We included multiple genetic targets in our assay based on analysis of SARS-CoV-2 genomes available. Finally, to avoid false-positive results, steps should be taken meticulously to prevent introduction of contaminating viral RNA or previously amplified DNA during preparation of nucleic acid extracts and amplification reactions.

**Conflicts of interest:** The authors declare that they have no conflict of interest.

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