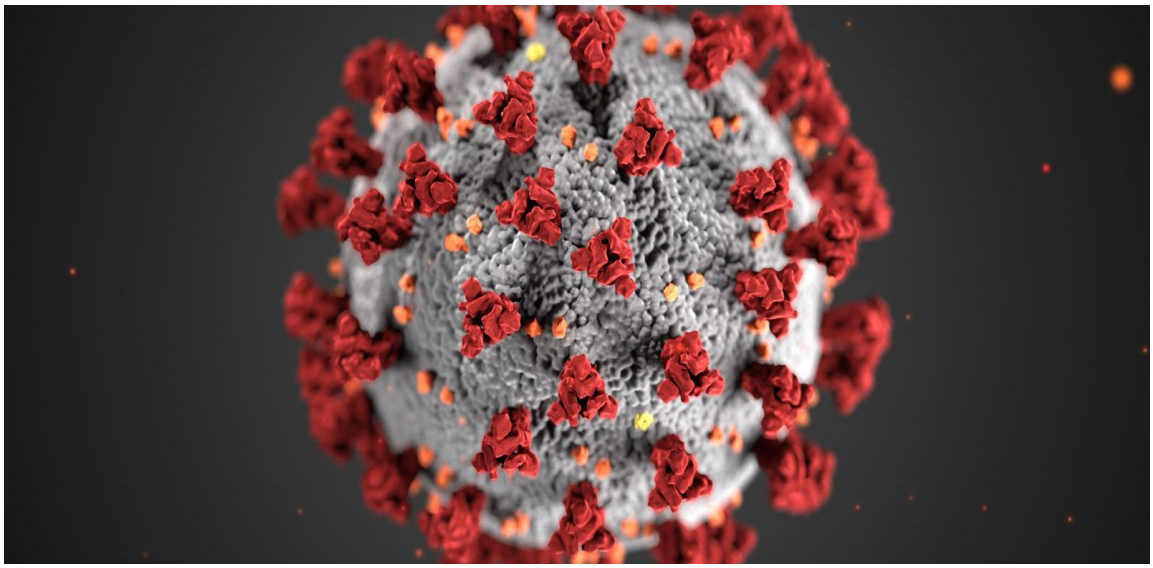
COVID-19

(A detailed report)



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1. Current Techniques used for Detection of COVID-19

a) COVID-19 RT-PCR test:

- **Introduction:** The COVID-19 PCR test mainly consists of a qualitative detection of nuclei acid released by SARS-CoV-2 in upper and lower respiratory specimen (such as nasal, nasopharyngeal or oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate or nasal aspirate). This test follows a real-time transcription polymerase chain reaction test from the samples taken from the suspected.

Results obtained implies the identification of SARS-CoV-2 RNA. Positive result indicates the presence of stated virus RNA sequence which is co-related with patient history and other diagnostic information to determine patient's infection status. Positive results do not rule out bacterial infection or co-infection with the viruses. The agent detected may not be the definite cause of disease.

Negative result contrarily doesn't imply the presence of SARS-CoV-2 infection but these results are combined with clinical observations, patient history, and epidemiological information to reach the final conclusion. A PCR test can confirm a diagnosis of COVID-19 if it identifies two specific SARS-CoV-2 genes. If it identifies only one of these genes, it will produce an inconclusive result.

- **Testing procedure, Principle used and Device description:**

Only the patients meeting the inclusion criteria based on the information provided on Pixel website are reviewed by Physical Wellness Network (PWN) which will determine test eligibility and prescription for the test. PAW will also follow up all positive and inclusive test results by contacting the patients. Negative patients are notified by email, phone message and through the website portal.

The collection kit contains a shipping box, pre-labeled return envelope, directions, specimen collection materials (nasal swab and saline tube), insulated specimen pouch, gel pack (for sample cooling) and specimen biohazard bag.

The COVID-19 RT-PCR Test is a real-time reverse transcription polymerase chain reaction (rRT -PCR) test. The test can be run in a single plex format (three individual assays) or multiplexed into a single reaction and amplification set up. In a single plex format, the test uses three primer and probe sets to detect three regions in the SARS-CoV-2 nucleocapsid (N) gene and one primer and probe set to detect human RNase P (RP) in a clinical sample. When multiplexed into a single reaction, the test uses two primer and probe sets to detect two regions in the SARS-CoV-2 N gene and one primer and probe set to detect RP. RNA isolated from upper and lower respiratory specimens (such as nasal, nasopharyngeal or oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate or nasal aspirate) is reverse transcribed to cDNA and subsequently amplified using Applied Biosystems QuantStudio7 Flex (QS7) instrument with software version 1.3. During the amplification process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5'nuclease activity of Taq polymerase degrades the bound probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle by QS7.

- **Instruments used:**

The COVID-19 RT-PCR test is to be used with the Roche MagNA Pure-96 (MP96) using MagNA Pure 96 DNA and Viral NA Small Volume Kit and Applied Biosystems QuantStudio7 Flex (QS7) instrument with software version 1.3 in a single plex format. When the COVID-19 RT-PCR test is multiplexed into a single reaction, it is automated on the Hamilton Microlab star liquid handler and uses two extraction methods: 1) Thermo Fisher MagMAX Viral/Pathogen Nucleic Acid Isolation Kit on the Thermo Fisher KingFisher Flex instrument; 2) MagNA Pure 96 DNA and Viral NA Small Volume Kit on the Roche MagNA Pure-96 (MP6), and Applied Biosystems QuantStudio7 Flex (QS7) instrument with software version 1.3.

Reagent	**Manufacturer**
DNA and Viral Small Volume Kit (3x192 purifications)	Roche
MagMAX Viral/Pathogen Nucleic Acid Isolation Kit	Thermo Fisher
TaqPathTM 1-Step Multiplex Master Mix (No ROX)	Thermo Fisher
COVID-19_N1-F Primer	IDT
COVID-19_N1-R Primer	IDT
COVID-19_N1-P Probe	IDT
COVID-19_N2-F Primer	IDT
COVID-19_N2-R Primer	IDT

COVID-19_N2-P Probe	IDT
COVID-19_N3-F Primer	IDT
COVID-19_N3-R Primer	IDT
COVID-19_N3-P Probe	IDT
RP-F Primer	IDT
RP-R Primer	IDT
RP-P Probe	IDT
COVID-19_N_Positive Control	IDT
Hs_RPP30_Internal Extraction Control	IDT

b) Serology Testing at CDC (Centers for Disease control and prevention)

- **Introduction:** Developed by CDC, it is a laboratory test to estimate the number of infected people in a country. Clinicians and researchers refer to this as a serology test, and many commercial laboratories call it an antibody test. CDC is using this serologic (antibody) test to evaluate the performance of commercial antibody tests. An antibody test looks for the presence of antibodies, which are specific proteins made in response to infections. Antibodies are detected in the blood of people who are tested after infection; they show an immune response to the infection. Antibody test results are especially important for detecting previous infections in people who had few or no symptoms.

We do not know if the antibodies that result from SARS-CoV-2 infection will provide someone with protection (immunity) from getting infected again. If antibodies do provide immunity, we don't know how much antibody is protective or how long protection might last. CDC scientists are currently conducting studies to answer these questions.

- **Testing procedure and Principle used:** Unlike viral direct detection methods such as nucleic acid amplification or antigen detection tests that can detect acutely infected persons, antibody tests help determine whether the individual being tested was ever infected—even if that person never showed symptoms. Serologic tests detect waning or past SARS-CoV-2 virus infection indirectly, by measuring the host humoral immune response to the virus. Therefore, serology assays do not typically replace direct detection methods as the primary tool for diagnosing an active SARS-CoV-2 infection, but they do have several important applications in monitoring and responding to the COVID-19 pandemic.

The two major antigenic targets of SARS-CoV-2 virus against which antibodies are detected are spike glycoprotein (S) and nucleocapsid phosphoprotein (N). While S protein is essential for virus entry and is present on the viral surface, N protein is the most abundantly expressed immunodominant protein that interacts with RNA. Multiple forms of S protein — full-length (S1+S2) or partial (S1 domain or receptor binding domain [RBD]) — are used as antigens. The protein target determines cross-reactivity and specificity because N is more conserved across coronaviruses than S, and within S, RBD is more conserved than S1 or full-length S.

- **Prominent types of antibody testing are:**

1. <u>Binding antibody detection</u>: These tests use purified proteins of SARS-CoV-2, not live virus, and can be performed in lower biosafety level laboratories (e.g., BSL-2). With specific reagents, individual antibody types, like IgG, IgM, and IgA, can be determined. In general, IgM is one of the first types of antibodies produced after infection and is most useful for determining recent infection, while IgG generally develops after IgM and may remain detectable for months or years. IgA is important for mucosal immunity and can be detected in mucous secretions like saliva in addition to blood, though its significance in this disease is still to be determined. Depending on the complexity of assays, these tests can be performed rapidly (less than 30 minutes) in a field setting or in a few hours in a laboratory.

2. <u>Neutralizing antibody detection</u>: FDA has not yet authorized the use of neutralization tests for SARS-CoV-2. Neutralization tests determine the functional ability of antibodies to prevent infection of virus in vitro. The test involves incubating serum or plasma with live virus followed by infection and incubation of cells. Testing will require either BSL-3 or BSL-2 laboratories, depending on what form of the SARS-CoV-2 virus is used.

- **Limitations of Serologic Tests:**

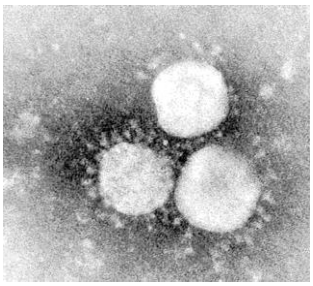
The kinetics of antibody response, longevity of antibodies, the ability of antibodies to protect from repeat infection, the protective titer of neutralizing antibody, and the correlation of binding antibody titers to neutralization ability are yet to be determined. Although animal challenge studies demonstrate protection in the short run, demonstration of long-term protection in humans will require future study. Hence, pending additional data, the presence of antibodies cannot be equated with an individual's immunity from SARS-CoV-2 infection.

Some tests may exhibit cross-reactivity with other coronaviruses, such as those that cause the common cold. This could result in false-positive test results. Some persons may not develop detectable antibodies after coronavirus infection. In others, it is possible that antibody levels could wane over time to undetectable levels. IgM and IgG antibodies are not present early in infection. Thus, serologic test results do not indicate with certainty the presence or absence of current or previous infection with SARS-CoV-2.

2. Virus Structure

Coronavirus virions (entire structure) are spherical to pleomorphic enveloped particles. The envelope is studded with projecting glycoproteins, and surrounds a core consisting of matrix protein enclosed within which is a single strand of positive-sense RNA (Mr 6×10^6) associated with nucleoprotein. The envelope glycoproteins are responsible for attachment to the host cell and also carry the main antigenic

epitopes, particularly the epitopes recognized by neutralizing antibodies. OC43 also possesses a haemagglutin.



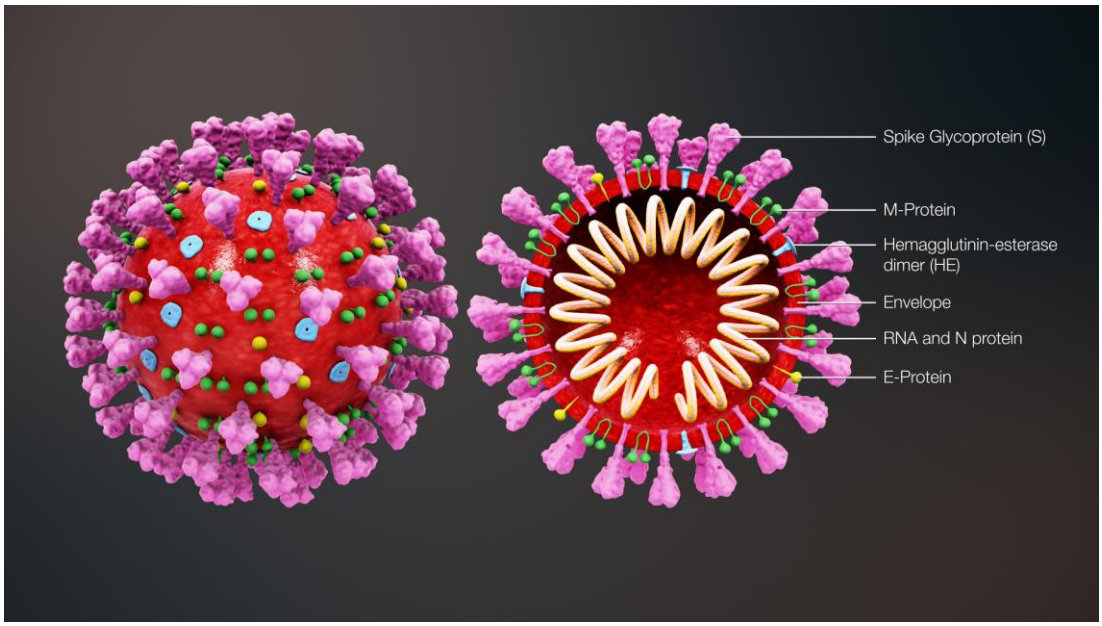
Electron micrograph showing human coronavirus 229E

Bar, 100 mn (Courtesy S.Sikotra, Leicester Royal Infirmary, Leicester, England.)

The viral envelope consists of a lipid bilayer, in which the membrane (M), envelope (E) and spike (S) structural proteins are anchored.[44] The ratio of E:S:M in the lipid bilayer is approximately 1:20:300. 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).

The coronavirus surface spikes are homotrimers of the S protein, which is composed of an S1 and S2 subunit. The homotrimeric S protein is a class I fusion protein which mediates the receptor binding and membrane fusion between the virus and host cell. The S1 subunit forms the head of the spike and has the receptor binding domain (RBD). The S2 subunit forms the stem which anchors the spike in the viral envelope and on protease activation enables fusion. The E and M protein are important in forming the viral envelope and maintaining its structural shape.

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. The lipid bilayer envelope, membrane proteins, and nucleocapsid protect the virus when it is outside the host cell.



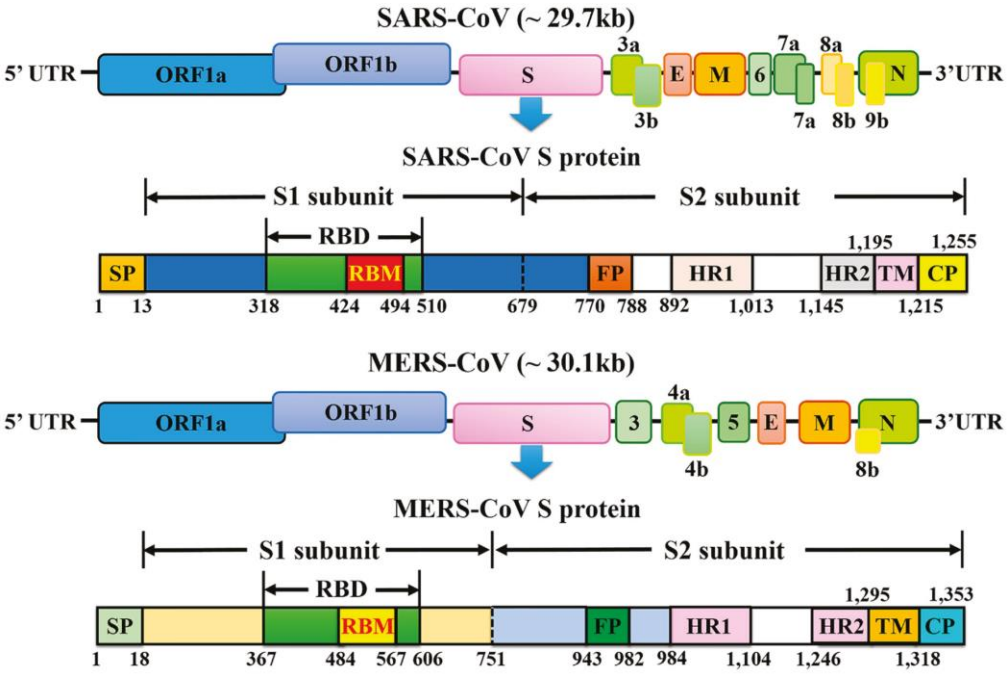
Cross-sectional model of a coronavirus.

Coronaviruses contain a positive-sense, single-stranded RNA genome. The genome size for coronaviruses ranges from 26.4 to 31.7 kilobases. The genome size is one of the largest among RNA viruses. The genome has a 5′ methylated cap and a 3′ polyadenylated tail.

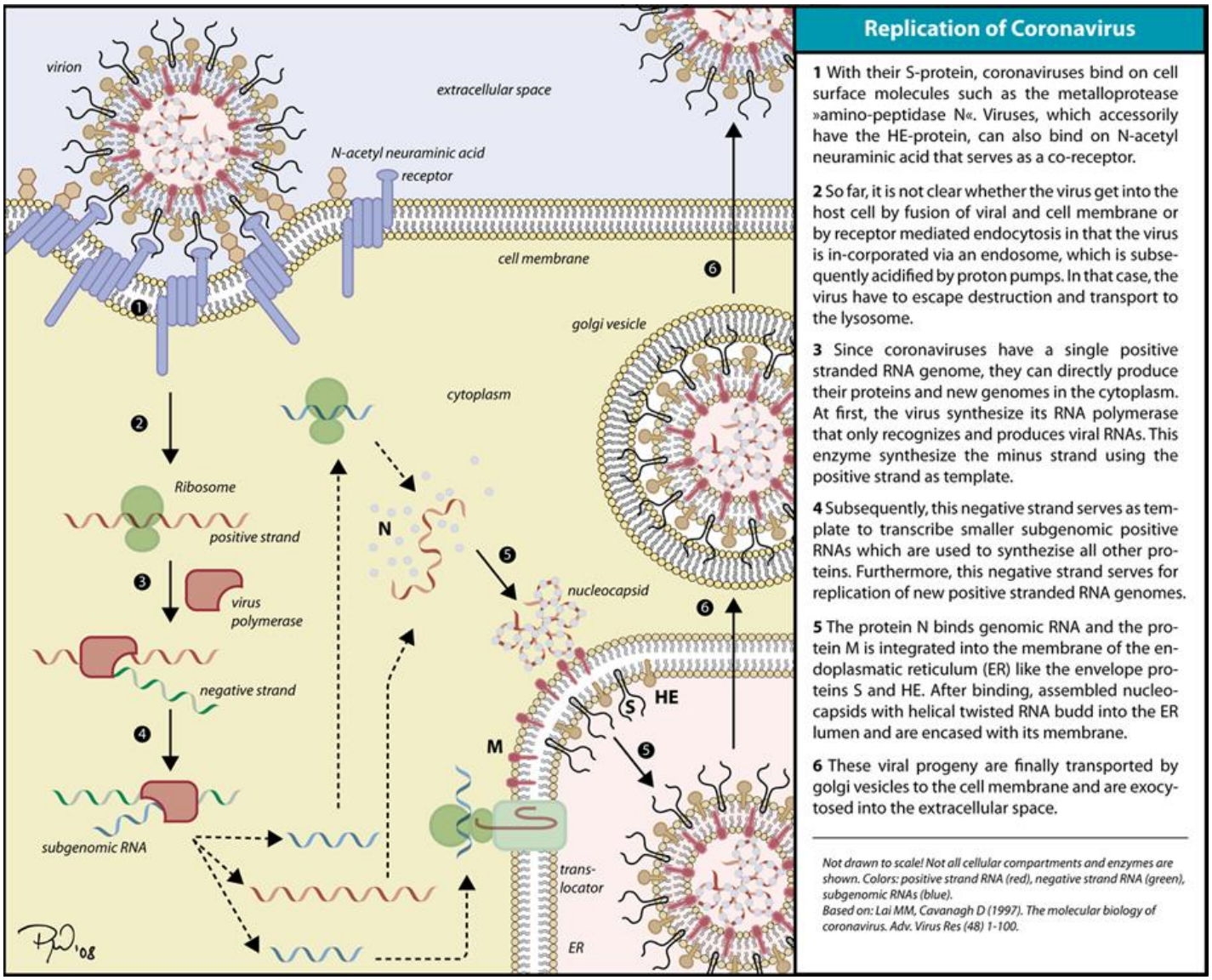
The genome organization for a coronavirus is 5′-leader-UTR replicase/transcriptase-spike (S)-envelope (E)-membrane (M)-nucleocapsid (N)-3′UTR-poly (A) tail. The open

reading frames 1a 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).

The later reading frames encode the four major structural proteins: spike, envelope, membrane, and nucleocapsid. Interspersed 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.



Schematic representation of the genome organization and functional domains of S protein for SARS-CoV and MERS-CoV



Life cycle of Corona Virus

3. Effect of Temperature on Corona

Abstract

The main route of transmission of SARS CoV infection is presumed to be respiratory droplets. However, the virus is also detectable in other body fluids and excreta. The stability of the virus at different temperatures and relative humidity on smooth surfaces were studied. The dried virus on smooth surfaces retained its viability for over 5 days at temperatures of 22–25°C and relative humidity of 40–50%, that is, typical air-conditioned environments. However, virus viability was rapidly lost (>3 $\log_{10}$) at higher temperatures and higher relative humidity (e.g., 38°C, and relative humidity of >95%). The better stability of SARS coronavirus at low temperature and low humidity environment may facilitate its transmission in community in subtropical area (such as Hong Kong) during the spring and in air-conditioned environments. It may also explain why some Asian countries in tropical area (such as Malaysia, Indonesia or Thailand) with high temperature and high relative humidity environment did not have major community outbreaks of SARS.

Introduction

The scientists have reported that infectivity of SARS CoV (SARS coronavirus) was lost after heating at 56°C for 15 minutes but that it was stable for at least 2 days following drying on plastic. It was completely inactivated by common fixatives used in

laboratory [9, 10]. Another study showed that it was inactivated by ultraviolet light, alkaline (), or acidic () conditions [11]. Human coronaviruses have been shown to survive in PBS or culture medium with 5–10% FCS for several days [12–14] but they only survive a few hours after drying [13, 14]. There have been some studies reporting an association between the SARS outbreak, metrological factors, and air pollution [15–17]. Thus, information on the survival of the SARS coronavirus (SCoV) in the environment at different temperature and humidity conditions is of significant interest to understanding virus transmission. A recent study using surrogate coronaviruses (transmissible gastroenteritis virus (TGEV) and mouse hepatitis virus (MHC)) has investigated the effect of air temperature and relative humidity on coronavirus survival on surface [18]. The survival effects of these environmental factors on SARS coronavirus remain unclear. In the present study, we report the stability of the SARS coronavirus at different temperatures and relative humidity.

Effect of Drying, Heat, and Relative Humidity

Ten microliter of maintenance medium containing 10^7 $TCID_{50}$ per mL of virus was placed in individual wells of a 24-well plastic plates and allowed to dry at room temperature (22~25°C) and relative humidity of 40–50% (i.e., conditions prevailing in a typical air-conditioned room). One hundred microliter of MM was used to resuspend the virus at 0 hr, 3 hr, 7 hr, 11 hr, 13 hr, 24 hr, and up to 4 weeks and the residual virus infectivity was titrated. Controls in closed screw cap Eppendorf tube were included each time and treated similarly but without drying.

The experiment was repeated at different temperatures (38°C, 33°C, 28°C) and relative humidities (>95%, 80~89%) for 3 hr, 7 hr, 11 hr, 13 hr, and 24 hr. A nebulizer under a controlled condition was used to generate high and relative low humidity environment. All the experiments above were conducted in duplicate and the residual viral infectivity was titrated.

Material and Methods

- **Virus Strain and Cell Line**

The SARS CoV strain used in this study is HKU39849. Foetal monkey kidney cells (FRhK-4) were cultured in minimal essential medium (MEM) with 10% foetal calf serum and penicillin streptomycin at 37°C in 5% CO_2 and were used for growing stock virus and for titration of viral infectivity.

- **Preparation of Stock Virus**

Stock virus was harvested when infection approximately 75% of the cell monolayer of a virus infected flask manifested cytopathic effect (CPE). Infected cells were subjected to one cycle of freeze and thaw centrifuged at 2000 rpm for 20 minutes to remove cell debris and the culture supernatant was aliquoted and stored at −80°C until use.

- **Determination of Tissue Culture Infectious Dose (50%) ($TCID_{50}$)**

96-well microtiter plates containing 100 μL of confluent FRhK-4 were infected with 100 μL of serial 10-fold of dilutions of stock virus in minimal essential medium with

1% FCS (maintenance medium) starting from 10^{-1} to 10^{8}. Titrations were done in quadruplicate. Infected cells were incubated for 4 days at 37°C. Appearance of CPE was recorded daily. $TCID_{50}$ was determined according to Reed and the Muench method.

Results

Ten microliters of 10^{7} $TCID_{50}$ per mL of virus was placed in individual wells of a 24-well plastic plate (representing a nonporous surface) and dried. The dried virus was then incubated at different temperatures (38°C, 33°C, 28°C) at different relative humidity (>95%, 80~89%) for 3 hr, 7 hr, 11 hr, 13 hr, and 24 hr and the residual viral infectivity was titrated. A similar experiment was conducted at room temperature and relative humidity of about 40–50% (air-conditioned room) for up to 4 weeks. Virus dried on plastic retained viability for up to 5 days at 22~25°C at relative humidity of 40~50% with only 1log(base10) loss of titre . After that virus infectivity is gradually lost every time. Loss of virus infectivity in solution was generally similar to dried virus under these environmental conditions. This indicates that SARS CoV is a stable virus that may potentially be transmitted by indirect contact or fomites, especially in air-conditioned environments.

4.Possible ways by which it can be killed

Each particle of the new virus, SARS-CoV-2, is studded with spikes, which allow it to attach itself to a human cell, poke a hole, and burrow inside. Like the germ that caused the SARS epidemic in 2003, it sticks to a protein on human cells called ACE2, which is especially prevalent in the lungs and small intestine. (SARS-CoV-2 is at least 10 times stickier than its cousin, which may account for its rapid spread.) One way to stop the invader is to keep it from attaching in the first place. This is what your immune system tries to do—it sends out antibodies that gum up the spikes so the virus can't stick to ACE2. But there are other ways of achieving the same effect.

1. Make a vaccine

For powerful, long-lasting immunity, a so-called live attenuated vaccine is the gold standard. It contains a defanged version of the virus that your immune system can use for target practice—but it can also cause infection. That's why many researchers are working on vaccines that contain not the whole virus but just the outer spikes. Mixed with immune-boosting molecules called adjuvants, they'll elicit a safe antibody response.

2. Take antibody-rich blood plasma

From people who have survived Covid-19 and inject it into newly infected or at-risk patients. Plasma won't teach the body how to fend off the virus, and one injection won't last forever—but it could be a good way to prepare health workers before they head to a hot spot.

3. Flood the zone with decoys

Synthetic molecules that look like ACE2 and trick the virus into binding with them instead, protecting lung cells from damage.

4. Invent drugs that hinder ACE2 from binding with the virus.

In theory, these compounds would work on both SARS and Covid-19, stopping the viruses from sticking to cells. But ACE2 plays a number of other roles throughout the body; it helps regulate blood pressure, kidney function, and even fertility. Messing with it could have dangerous consequences.

Kill It on Contact

All viruses wear heavy-duty protein coats to protect their precious genetic material from the elements. The new coronavirus sports an extra outer layer of fatty molecules. That's great news for humans, because it's easy to tear open with soap or alcohol-based disinfectants. (Soap works best, and you don't need to bother with the antibacterial stuff.) Without its fatty layer, the virus dies. Wipe it away or wash it down the drain.