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A REVIEW PAPER ON THE CURRENT DIAGNOSTIC APPROACHES FOR THE SARS-CoV-2

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Abstract

COVID-19 is an evolving phenomenon. Originated from a cluster of patients with violent yet similar symptoms of respiratory illness as of viral flu in Wuhan, China late-December 2019, which later confirmed as a novel strain of Beta-coronavirus, illustrating transmitted from bats to humans with an undefined indication of any intermediate host.SARS-CoV-2 is transmitted by respir¬atory droplets and fomites and present clinically with fever, fatigue, myalgia, conjunctivitis, insomnia, dysgeusia, sore throat. However, some with escalated symptoms into acute respiratory distress syndrome accompany inflammatory cytokines response and multi-organ failure. The virus spread it spikes to international borders with extraordinary ferocity and speed stretching its course with an explosive increase in death tolls from March from a few hundred to a hundred thousand to crossing 50 million as of now. The wide range in the severity of the infection makes it difficult to access the overall infection rate. For that, an immense need for rapid and accurate diagnostics methods to better prevent the spread of COVID-19. For the testing, CDC recommends, two kinds of tests are available for COVID-19. First, a viral test tells you if you have a current infection. Second, an antibody test tells you if you had previous infections. The present review discusses the current literature on the modalities, including nucleic acid Amplification Tests(RT-PCR), Direct Viral Antigen tests, and other serological antibody-based tests with varying accuracy and efficacy highlighting various future approaches to enhance the sensitivity of the test and lowering the false positive outcomes.

KEYWORDS: COVID-19, SARS-CoV-2, RT-PCR, serological, amplification, CDC

Introduction

This review aims to evaluate the current diagnostic methodology available for COVID-19. The article will begin with the emphasis on the origin and variant strains of SARS-CoV and how it led its path to COVID-19 describing its virology, current epidemiology studies, and various test methods from RT-PCR to antigen-based serological tests. It will look into various future approaches to rapid testing and increasing the efficacy and sensitivity.

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HISTORY OF CORONAVIRUSES

Coronaviruses are a large family of enveloped, non-segmented, single-stranded, positive-sense RNA viruses that circulate among animals including camels, cats, and bats. When observed under an electron microscope, coronaviruses have a crown-like appearance owing to the presence of spike glycoproteins on their envelope that infects humans along with a wide range of animals. In 1966 by Tyrell and Bynoe presented a detailed explanation of coronaviruses after cultivating viruses from patients suffering from common colds.

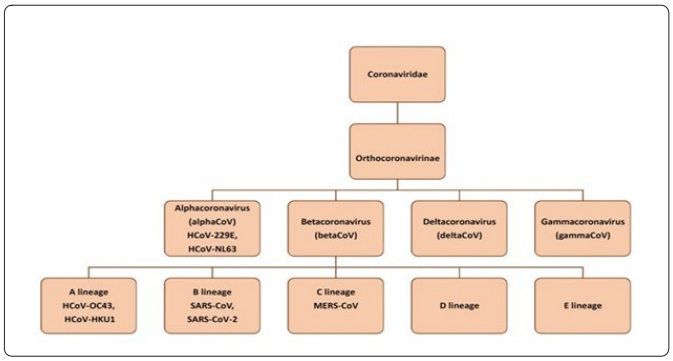
Six strains of coronavirus have infected humans, four of which are together responsible for about one-third of common colds. In the past two decades, there have been three global coronavirus outbreaks. Beginning with the Severe Acute Respiratory Syndrome (SARS), caused by a coronavirus called SARS-CoV, occur in 2003 in Guangdong, China, and later spread its legs to many countries in Southeast Asia, North America, Europe, and South Africa. Bats are the natural hosts of SARS-CoV; its intermediate hosts are palm civets and raccoon dogs. Early cases of SARS were linked to human and animal contact at live game markets. Transmission occurred person-to-person through droplets produced by coughing or sneezing, via personal contact, and by touching contaminated surfaces. In SARS, peak viral shedding occurs approximately 10 days after the onset of illness, when many patients are hospitalized, which explains why health care professionals have a particularly high risk of becoming infected. SARS-CoV has an R0 of 4, meaning that each infected person spreads the disease to an average of four o others, and a case fatality rate of 9.5 percent. Although the virus-infected 8,069 persons and caused 774 deaths, the last known case of SARS was detected in September 2003. Nine years later, MERS-CoV – which causes Middle Eastern Respiratory Syndrome (MERS) – emerged in Saudi Arabia. MERS is characterized by sporadic zoonotic transmission from camels and limited episodes of person-to-person transmission. Explosive nosocomial transmission has been linked to single super-spreaders of infection. Almost all cases have been linked to people in or near the Arabian Peninsula. However, the symptoms of MERS are nonspecific, but many patients develop atypical pneumonia and severe acute respiratory distress. Additionally, patients often have prominent gastrointestinal symptoms and acute kidney failure. This constellation of symptoms is due to the binding of the MERS-CoV S glycoprotein to dipeptidyl peptidase 4, which is present in the lower respiratory tract, gastrointestinal tract, and kidney. The disease is still circulating and, to date, has infected approximately 2,500 people and caused 850 deaths. The main factor that controls the spread of MERS-CoV is its very low R0 of 1

CLASSIFICATION OF CORONAVIRUSES

Based on their morphological structures as spherical visions with a core-shell and projections on the surface of enveloping that look like a solar corona, these viruses were called coronavirus (Latin: corona = crown). The Coronaviridae family (order Nidovirales) diverges further into subfamilies of which subfamily Orthocoronavirinae further classified into four genera of CoVs: (AlphaCoV), (betaCoV), (deltaCoV), and (gammaCoV)

Furthermore, the betaCoV genus is divided into five sub-genera, also called lineages. Interestingly, alpha and beta have gene sources of bats

and rodents, while delta and Gamma resemble avian species. Among them, 7 human CoVs can infect the human, of which Beta is known to cause severe illness a potentially deadly epidemic and pandemic condition? This human CoV (HCoV) include HCoV-OC43, HCoV-HKU1, HCoV-229E, and HCoV-NL63. In terms of severity, their action is restricted to the common cold and upper respiratory infection in immune-competent individuals whereas it might lead to serious lower respiratory infection in immune-compromised patients.

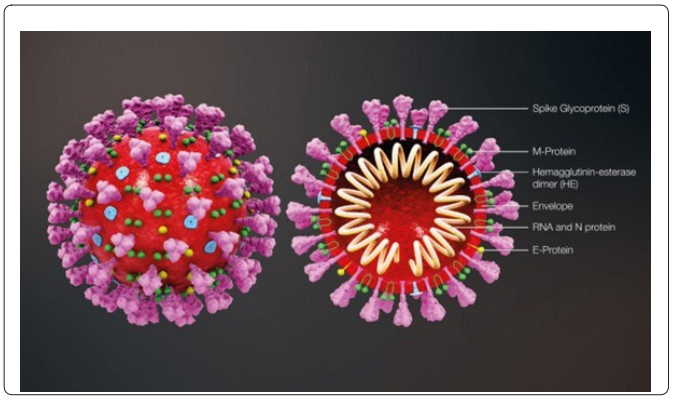


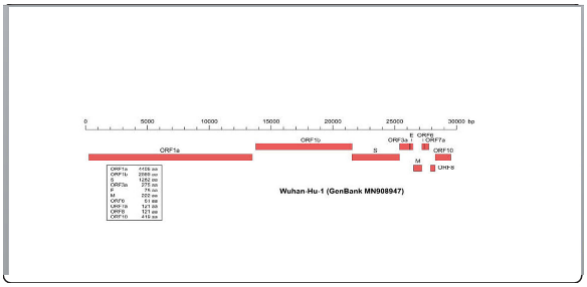
ORIGIN AND OUTBREAK

The horror began at the late end of December 19, 2019, in Wuhan, a capital city of Hubei province, a large transportation hub in the world's largest populated country, China when a bunch of people admitted themselves to local hospitals with pneumonia of unknown etiology. Some of the initial cases had been exposed to the Huanan wholesale seafood market having trade of live animals. After many patients showed up at with unknown cause, the surveillance system took their respiratory samples of patients and sent for further analysis. On the dreadful day of 31 December 2019, the same was reported to the World Health Organization (WHO)'s China bureau in Beijing. By January 2, 2020, the full genome of a new coronavirus (SARS-CoV-2) had been sequenced by Shi Zhengli, a coronavirus expert at the Wuhan Institute of Virology; just over a week later, the sequence had been published and the Chinese National Health Commission warned of its potential danger. The virus was initially referred to as "novel coronavirus 2019" (2019-nCoV) by the WHO – but, on February 11, 2020, was given the official name of SARS-CoV-2 by the International Committee on Taxonomy of Viruses. On 1st January the Huanan seafood market was closed. On 7th January the virus was identified as a coronavirus that had >95% homology with the bat coronavirus and>70% similarity with the SARS-CoV. Environmental samples from the seafood market also tested +Ve signifying that the outbreak originated there. However, the number of cases started increasing exponentially, some of which did not have exposure to the live animal market, suggesting that there is a human-to-human transmission. The direst fatal case was reported back in Janurary11, 2020. The abrupt migration of Chinese citizens during the Chinese New Year added fuel to the fire and instigated an epidemic that eventually ends up consuming a major part of the country itself and crossed the south-Asians boarders engulfing countries like Thailand, Malaysia, South Korea. By 23rd January, the Wuhan was placed under lockdown. Airports in different countries including India put in screening mechanisms to detect symptomatic people returning from China and placed them in isolation and testing them for COVID-19. Soon it was apparent that the infection could be transmitted from asymptomatic people and also before the onset of symptoms. Therefore, countries including India who evacuated their citizens from Wuhan through special flights or had travelers returning from China, placed all people symptomatic or otherwise in isolation for 14 d and tested them for the virus. Furthermore, on the 12th of February, China burst with an increase in cases by 15k/day. By the onset of March 2020 havoc of this virus emerged worldwide with 96,000 cases (80,000 in China) and 87 other countries and 1 international conveyance (696, in the cruise ship Diamond Princess, parked off the coast of Japan) have been reported. Quite surprisingly, the number of cases declines tremendously afterward in Host country China, however, the rest of the world experience its first deadliest Pandemic in the face of the earth with a dramatic surge in a widespread exponential increase in cases from in thousands in April 2020 to hundred thousand (millions) by September 2020.

VIROLOGY AND STRUCTURE

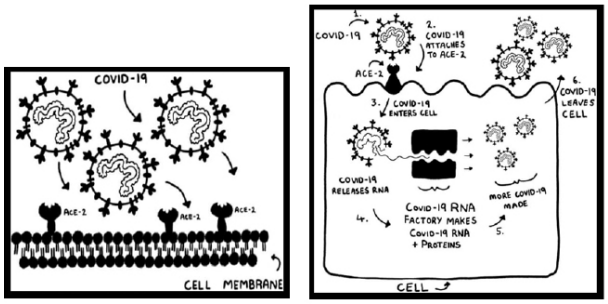
SARS-CoV-2 is a beta-coronavirus (an enveloped, single-stranded RNA virus) with genetic sequence homology of 96% with the RATG13 coronavirus strain in bats and 79% with the SARS-CoV. On the contrary of bat coronavirus, SARS-CoV-2 has a spike protein with high-affinity binding interaction to human ACE2 receptors which are present in many body parts and tissues (lungs) and functional polybasic cleavage site at the junction of the spike protein's S1 and S2 subunits which is a feature that enhances spike protein cleavage and increases viral infectivity of the virus. The RNA genome consists of 29,903 nucleotides – larger than most other RNA viruses. One-third of the genome consists of genes for the four structural proteins and eight genes for accessory proteins that inhibit host defenses. Most of the remainder of the genome consists of the replicase gene, which encodes two large polyproteins that are cleaved into 16 nonstructural proteins (NSP) that assist in replicating and proofreading the viral genome.





BINDING INTERACTION

Our body has evolved numerous defense to protect us against various pathogens. However, the bad news is that our body has a fatal flaw. on the surface of many of our cells, there is an enzyme called ACE2. These ACE2 enzymes play an important role in controlling our blood pressure. Some of the most popular drugs used to control blood pressure are called an ACE inhibitor. They do this by binding ACE2 on the surface of blood vessels, which causes them to relax, hence lowering the BP. Interestingly, with an unknown mechanism, the spikes on the surface of the COVID-19 virus are just hs the right shape to lock on these ACE2 enzymes and bind tightly acting like a popular lock and key fitting. Eventually, that led to the opening up of the human cell membrane and slippage of the virus inside. Evading the virus causes the immune system to spring into action and unleash hell. But in the case of COVID-19, it has evolved a mechanism to bypass the detection by the host immune system which is equivalent to cutting the telephone lines, silencing the alarm before they go off.



LITERATURE

A literature search was carried out for the underlying study on the 'the Great COVID-19'-The description of the Deadliest Pandemic in History of Mankind that primarily focused on the diagnostic and serological testing for SARS-CoV-2/COVID-19, using the keywords; "coronavirus" / "COVID-19" / "SARS-CoV-2". For the following review paper, data and article published was retrieved from the sources like NCBI, PubMed, and Google Scholar from inception through January 2020 to October 2020. Additional journal articles were identified from the bibliographies of included studies. The protein and virus structure details the latest was retrieved from the structure database. For the main objective of this review, the latest and approved test with valid sensitivity and specificity against COVID-19 was included in the study. More than 20,000 articles have been published on SARS-CoV-2, of which 4182 articles are related to COVID-19 and antibodies or serology tested out of which the latest methods approved for the physicians to diagnose have been studied and reviewed. The guidelines and diagnostic measures approved by the WHO and CDC are also be taken care of.

Sources: Dr. Matthew B. Frieman and Dr. Stuart Weston, Univ. of Maryland School of Medicine; Fields Virology; Fenner and White's Medical Virology; Nature; Science; The Lancet; New England Journal of Medicine; Centers for Disease Control and Prevention.https://www.nytimes.com/interactive/2020/03/11/science/how-coronavirus-hijacks-your-cells.html

DIAGNOSTIC METHODS

By May 2020, Foundation for Innovative New Diagnostics, which is the WHO Collaborating Centre for Laboratory Strengthening and Diagnostic Technology Evaluation, contained 560 SARS-CoV-2 laboratory tests for the diagnosis of COVID-19. These comprised 273 molecular assays and 287 immunoassays. Excluding those intended for research use only, 152 of these are molecular assays and 211 immunoassays are CE-marked for in-vitro diagnostic devices. However broadly speaking, there are majorly two types of tests available for COVID-19: viral tests and antibody tests. The former detects the virus and indicates the current infection, on the contrary, the latter instead of detecting the virus, indicating the previous exposure to the infectious virus.

Amplification based diagnosis

As soon as after the outbreak of pneumonia-like etiology was detected in Wuhan, On January 11, 2020, the SARS-CoV-2 genome was sequenced and published by China. Immediately after that within a week, a virologist from Berlin, Germany came up with the first-ever RT-PCR (reverse transcriptase real-time polymerase chain reaction as a diagnostic tool for COVID-19 which was later approved by WHO and issued for practice under immediate effects. However, on one hand, many countries opted for the RT-PCR tool kit, unfortunately, the US's CDC Refused it initially and ordered to produce its kits which later turned out to give many false positive and unreliable results. Eventually, CDC publishes the standard protocols, primers, and probes. The US Food and Drug Administration (FDA) issued new guidance on February 29, 2020, so that labs could develop and use COVID-19 molecular diagnostic tests (but had to apply for Emergency Use Authorization, or EUA, within 15 business days of clinical use). Although clinical labs could purchase primers and probes for the CDC assay from Integrated DNA Technologies (IDT), other reagents had to be procured elsewhere.

With a genome of 29,881bp, ssRNA. The foremost aim of standard PCR is to amplify a particular gene of interest with a small amount of template DNA and Primers specific to the gene of interest are then undergoes repeating cycles of heating and cooling steps and amplify the gene into millions of copies. However, in its variant type RT-PCR, the starting material is RNA, which is reverse-transcribed into complementary DNA (cDNA) that serves as a template in the PCR reaction. In our case, the virus is an RNA virus, so RT-PCR has been used for detection by providing the result in 2-3 hours. However, to do so firstly Nasopharyngeal (NP) is taken which if not inserted properly might give false +ve. The NP samples then send to the lab in saline media stored at 2-8°C. Next, follows the standard RT-PCR Protocols, including cell lysis, nucleic acid extraction, and purification, and multiplexed PCR amplification and detection with fluorescence signal readout. However, as many of these tests came out quickly to help initially boost COVID-19 testing, all of the tests with FDA-EUA status are still only qualitative, giving a dichotomous indication of either presence or absence of SARS-CoV-2 without quantifying viral load.

Target Interaction in RT-PCR

The WHOs RT-PCR assay primarily targets the SARS-CoV-2 envelope gene and the RNA-dependent RNA polymerase gene. If both targets are detected, the result is +ve. But if only one is detected, the result is concluded as inconclusive. In the assay of CDC, they focused particularly on 3 different amplification regions of N gene

- NS3 – which detect all SARS-like coronavirus

- N1 & N2 – only specific for SARS-CoV-2

Furthermore, many researchers and pharmaceutical companies designed RT-PCR Assay targeting regions like ORF, Envelope Nucleocapsid, and RNA-Dependent RNA polymerase genes

Recent studies indicate that SARS-CoV-2 viral load peaks in the first five to six days of disease onset. Viral RNA can be detected during the second week of disease onset, but the viral load is lower. If repeat PCR testing is warranted, the second specimen should be performed 24 hours after the first collection; longer intervals between specimens increases the risk of missed diagnosis because viral load decreases with time. Repeat testing of a lower respiratory sample (Bronchoalveolar lavage) might be necessary for the diagnosis of a patient with severe or progressive disease and repeat negative results with nasopharyngeal swabs.

ANTIBODY BASED DIAGNOSIS

Serologic tests detect antibodies that form in blood after SARS-CoV-2 infection. Most of the tests flooding the market are lateral flow assays; laboratory-based antibody tests are either enzyme-linked or chemiluminescent immunoassays.

What this test does is it Ab to two SARS-CoV-2 proteins i.e. either spike protein or nucleocapsid phosphoprotein. However, Enzyme-linked and chemiluminescent detect either total Ab, IgG alone of both IgG and IgM separately, and lateral flow assay check for IgG and IgM Ab separately. Also, it is suggested not to consider the result of the antibody test alone to diagnose for COVID-19 infection. It is advisable to couple this with RT-PCR since patients with symptomatic COVID-19 do not have detectable Ab to SARS-CoV-2 within 10 days of symptoms onset. Even patients with confirmed viral RNA through RT-PCR have detectable IgG 14days after symptoms onset so this will eventually lead to the omission of the infectious patients in an early stage of the disease and those with mild symptoms having lower Ab titers. Likewise, happen with immunocompromised patients

Currently, no one knows how long antibodies to SARS-CoV-2 persist. Seasonal coronavirus antibodies decline only a few weeks after infection and some people are susceptible to reinfection within 1 year. Nevertheless, In SARS-CoV Ab's peak approximately 4 months after infection and protect patients for 2-3 years.

SUMMARY

In this review, we have attributed various aspects of the current pandemic with its origin and various strains, describing potential outbreak and prior warning. In regards to diagnostics methods for detection of the presence of SARS-CoV-2 discussed hereby the two broad principle techniques in practice: Nucleic Acid Amplification Test and Antibody Testing. RT-PCR is done by taking swab samples. The most common swab types are nasopharyngeal swabs and oropharyngeal swabs. Then comes the antibody detection through serological assays, most commonly ELISA, in which SARS-CoV-2 specific IgG and IgM antibodies are detected to measure general immunity to SARS-CoV-2. This is important not only to examine the infection severity and chance for successful recovery in an individual but also to determine if herd immunity has been reached for an overall population. Future work in this field will include quantitative testing approaches in nucleic acid and antibody/ antigen assays and the development of a SARS-CoV-2 specific genetic signature.

CONCLUSION

Few of us have lived through anything like this or had to face such an uncertain future. The world had plenty of warnings that an unstoppable pandemic was not only possible but highly likely. The WHO has pointed out again and again that it was only a matter of time before something like his happen and now it is here. Amongst us .like a terrifying science fiction story which has suddenly become real. This deadly outbreak of the virus has scum mankind and has shaken the economic, medical, and public health infrastructure worldwide. Only time will tell how long this will last. However, there is guardedly optimistic that science will come to rescue, and apart from curbing this outbreak, efforts should be made to devise comprehensive measures to prevent future outbreaks of zoonotic origin and will develop more accurate tests and more effective treatments as well as importantly 'vaccine'.

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