Covid-19, History and Modern Laboratory Diagnostic Techniques: A Rapid Review

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Abstract

The great catastrophe that the world faced after World War II, which is the Corona epidemic, was discovered in the 2019 year in Hubei Province, Wuhan, China, and it is a severe respiratory disorder. The World Health Organization gave it this name, and it is one of the viruses (SARS-CoV-2). Among the problems that the world faces is how to control them. Given the seriousness of the disease, we need to find quick ways to limit the spread of the virus. The reverse transcription-polymerase chain reaction (RT-PCR) test is the current gold standard diagnostic method for the diagnosis of COVID-19 and is considered one of the modern techniques until now for managing the current outbreak to detect SARS-CoV-2 infections. However, several other faster and more accurate tests are being developed for the diagnosis of SARS-CoV-2 aiming to control the spread of infection through the identification of patients and instant isolation of the virus.

Keywords: Covid-19, RT-PCR, Lab Diagnostic Techniques, Hot-Start.

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INTRODUCTION

The beginning of virus SARS-2 began at the November of the year 2019 in the Chinese city of Wuhan 1. Many scientists have declared that there are two theories for the exit of the virus; 1-The virus evolved into a pathological clarify after forming any changes to it in scientific laboratories, in a non-human host, and transferring to a human host, 2- The spread of the virus from a non-human host to a human host led to its not noticed development in the human body 2,3.

Coronavirus (SARS-CoV-2) is an enveloped virus and contains spike proteins surrounding the envelope (S) that become strong to attach to receptors on the surface of cells and begin the invasion process 4. The emergence of the first virus was in December of the year 2019 5, but in January 2020 where the case was found in Tibet, which led to the spread of the virus, but on 31 January the case was from the Chinese mainland, and thus it continued to spread, causing human astonishment and panic in the whole world and many fears it generated from this virus. That led to the hesitating of development and even led to the destruction of the economy of many countries due to the inability to manage it and this is what prompted the world and the World Health Organization to declare a global emergency against the virus until it is possible to control and eliminate it 6.

Given the importance of early and rapid detection of Covid-19, many faster and more accurate tests are being developed to diagnose SARS-CoV-2 that would control the spread of infection by quarantining patients and immediately isolating the virus.

In this review, we seek to highlight the modern laboratory diagnostic techniques being developed for the diagnosis of Covid-19 aiming to control the spread of infection through the identification of patients and instant isolation of the virus. So, the development of rapid and reliable techniques for COVID-19 diagnosis is a significant step to prepare for any pandemic in the future.

Methods

A thorough literature search was done for three months to extract relevant papers for this rapid review study. Research papers in English from June 2020 to May 2022 were studied from databases such as EMBASE, MEDLINE, Google Scholar, Cochrane Library (Wiley), PubMed, and Web of Science.
A combination of keywords such as 'Covid-19', 'SARS-CoV-2', 'RT-PCR', 'Lab Diagnostic Techniques', 'Hot-Start', was searched. The extracted papers were then inspected for relevance to the review topic, and subsequently, papers relevant to the topic were included. Out of 68 articles collected on the topic of research, 35 papers were read for final review writing after discarding 14 irrelevant papers.

Human Cells and The Mechanism of Their Destruction
At the beginning of infection with the virus, it takes place through receptors on the membrane of the receiving cell on the surface of the virus. Where the proteins of the virus have been identified and then it attacks the cell through the method of cell endocytosis mediated by clathrin 7. After binding, the virus reaches an organ called the endosome, knowing that there are two ways for the virus’s DNA to exit into the cytoplasm: 1. The viral envelope and DNA are removed and go to the cytoplasm; 2. Interference of the particle with the lysosome of the host cell. The enzymes by dismantling the protein cover, and then entering the virus genome into the cytoplasm of the cell DNA polymerase 8 which is present in the coronavirus, consisting of a 5-methylation. It is presented at the 5th end of the RNA. The enzyme RNA polymerase depends on translation from RNA) to new RNA via 3 to 5. Replication represents the formation of RNA with different types and then binds to ribosomes to become active and make new copies, the cell loses its function and explodes to release the virus to other body cells 9.

Virus and its spread
At the beginning of the outbreak of the virus, it is necessary to know the ways leading to its spread. Variation in the means of transportation over time is one of the causes of the epidemic situation where the virus is considered a growing virus. It appears similar to SARS and Corona in the past. Bats may be considered a source of the virus 10. The virus caused the spread of acute lung disease in China at the beginning 11 and its transmission appeared from person to person. The main ways of transmission are respiratory droplets, direct or indirect contact with these drops and the incubation time is (2-5) days 12. Co-infection of COVID-19 with other viral diseases are well-known. SARS-CoV-2 with co-infections such as the human immunodeficiency virus (HIV), HBV, and HCV, are widespread in patients 13.

Methods For Isolating The Virus in vitro
The virus is isolated and diagnosed by sequencing the genome of Covid-19 by polymerase chain reaction technique, where the diagnosis depends on the quantitative polymerase chain reaction to detect the DNA of Corona virus 14,15.

RT-PCR
This technique is considered one of the modern techniques for managing the current outbreak 1, where studies have shown the use of samples from the respiratory system in the examination 14 to detect the presence of the virus or confirm that the virus is not present 16. The technique used to determine the amount of DNA or RNA in the sample, where the real time in which DNA doubling occurs in this technique is indicated, and it is represented in three stages: 1-Denaturation, 2-Annealing, and 3-Extension. The first stage takes place at 94 degrees Celsius, where the DNA is de-duplicated to become one strand of DNA, then the second step is represented by the annealing of the primers at a temperature of 55 degrees Celsius, where the primer binds to the single strand as well as the fluorescent dye used or the probe used in the process. As for the third step, it is represented by stretching at a temperature of 72 degrees Celsius. The enzyme DNA Taq is activated, as it works at high temperatures and works to add the desired bases to the single DNA strand to complete it.

Components Used into the Real-Time PCR
It also consists of bases that the enzyme works to add as supplements to the tape and the addition is done by the action of the enzyme DNA Pol, where the enzyme is considered highly effective in high degrees, as well as MgCl2 where it has a role in amplification, the concentration (MgCl2) differs from the traditional (PCR) concentration, which is (cofactor) for the enzyme as the concentration of the template. It is different if (RT-PCR) samples are placed in the device and after amplification, a standard or quantitative curve is performed. Therefore, there is no need to migrate with agarose. RT-PCR is considered accurate
and sensitive. After each interaction, it will emit fluorescence, which will be detected and quantified as an indication of the amount of the virus.

Melting curve analysis
After the amplification reaction has finished and the fluorescence signals have been captured, the template is melted to determine non-specific binds. The dye dissociates and the fluorescence signals are diminished when the template is melted with heat. "Larger sequences take longer to melt and have a higher melting temperature, but non-specific bands melt faster and have distinct melting temperature curves." The data is represented in a graph, which shows fluorescence vs. melting temperature (Figure-1). A dissociation curve is a graph of fluorescence versus melting temperature, and a dissociation curve analysis is a method for analyzing 17.

![Normalized Melt Curve](image)

**Figure 1:** Summary of genotyping presented on normalized and difference melting curve plot, fluorescence expressed in relative fluoresce units (RFU). Three melting curve clusters for samples from manual DNA isolation method.

The value of Ct
Ct value is the point at which fluorescent shine appears, indicating the beginning of replication. The cDNA denatures, the primer anneals, and the template expands in a cyclic denaturation, annealing, and expansion process known as RT-PCR. The machine amplifies a template nucleic acid in a cycle if it is present; if it is not present, the cycle stops. The catch is that if 30 cycles pass without nucleic acid amplification in the remaining 31 cycles, the sample's definite Ct value is 30. In this circumstance, 35 to 40 cycles are completed, resulting in a lower viral burden. Amplification in the 15th cycle indicates that the sample has a greater viral load. If no viral particles are present in a sample, no amplification will occur in all 40 cycles in the usual situation.

The Normal Ct value
There should be 38 to 40 cycles of RT-PCR amplification with positive and negative controls. Ct values of 38 to 40 are considered typical. Based on the severity of the disease and viral load, infected samples or samples with a positive COVID-19 infection value are separated into two categories. • If the Ct value is less than 29, the sample is infected with SARS-CoV-2 or has a high viral load. • A Ct value of 30 to 37 suggests a moderate to mild infection, and a Ct value of 30 to 37 indicates a moderate to mild infection. Higher viral load samples multiply from the start of the cycle to the end, resulting in a very high infection.
Hot Start
HotstartTaq. The Hot Start differs from the standard Taq DNA polymerase in several ways. Chemical modifications have been made to every commercially available Hot Start Taq. Taq DNA polymerases with a quick start are either, 1. enzyme-linked, 2. oligonucleotide-linked, or 3. an inert enzyme that has been chemically changed.

One of the most successful variations of the classic PCR procedure for generating the most exact and trustworthy results is the Hot-start PCR. The hot start PCR was created with the intention of improving the reaction's performance. One of the most successful methods for identifying inherited disorders is this one. It all starts with a high fever. Once the PCR process has reached the heating step, this DNA polymerase is activated. In this scenario, the DNA polymerase is linked to an antibody.

Mechanism of action
The fluorochrome is released from the lengthy chain of the single-stranded probe, resulting in fluorescence that the detector detects. In contrast, the primer binds to its complementary sequence at the precise annealing temperature. Taq DNA polymerase begins adding dNTPs towards the 3’ end of the strand after annealing until it reaches the end. After the procedure is completed, a new DNA m is created.

Type of Controls PCR
Negative, Positive and Internal Controls are common PCR control types employed as a results.
Controls has two types: 1. Negative and Positive controls. Positive control can be sub-divided into: a. internal control, b. external control, c. reaction controls.
• Negative control: The lack of template DNA in these controls is referred to as a negative control, implying that we do not contribute template DNA to the reaction throughout its preparation. The end results in the negative control show nothing. Instead of the template, nuclease-free water was utilized in the negative control. Negative control is used to supply us with information about. Whether or whether the reagents are tainted.
The positive control can be divided into:
True positive control
Internal control
Standard control
External control
  ■ All of the materials, including Taq DNA polymerase, PCR reaction buffer, dNTP mix nuclease-free water, and a particular template, are produced in a separate external positive control tube. A lack of amplification implies a problem with the reaction's preparation. It tells us if our polymerase is operating well and whether all of the components are being used correctly.
  ■ Internal control:
Internal positive control is more important than external positive control. It evaluates the precision and quality of performance at a certain response level. It also tells you whether each PCR component is operating correctly. It tells us if our polymerase is operating well and whether all of the components are being used correctly.
  ■ Standard control:
Standard control is nothing. It actually can’t provide data regarding if the reaction occurred well or not rather it gives an idea about if there is something wrong with the gel preparation or not.

Detection of the virus via Serology
SARS-CoV-2 antigen recognition reagents Immunoglobulin M (IgM) and IgG discovery reagents, as well as colloidal gold and catalyst-linked immunosorbent technologies, have recently been successfully created and used to help in the identification of SARS-CoV-2 18.
Crispr
The advancement of nucleic acid detection technology has been boosted by CRISPR technologies. Using CRISPR enzymes’ unique enzymatic capabilities, researchers created a revolutionary nucleic acid detection method that allows for multiplexed quantitative and extremely sensitive detection, as well as lateral visual readout flow 19.

Techniques of Radio Imaging
Because interpreting chest X-ray data is difficult, various imaging methods must be examined. Patients are still inadequately positioned and may have preexisting comorbidities that confound interpretation, despite the fact that a chest x-ray is simple to get, compact, and interpretable. CT scanning, on the other hand, has improved lung specificity and visibility when researching. A CT chest scan can reveal the full extent of the lung invasion. On CT scans, most COVID-19 patients show similar features, such as bilateral dispersion of aberrant colors and ground glass opacity 20. The CT patterns were discovered in a number of people who had been "suspected" but had tested negative for SARS-CoV-2 viral RNA 21. The data was interpreted using artificial intelligence.

Guidelines/Preparedness Plans for a Covid-19 Pandemic from the WHO
In metropolitan centers, towns, contemporary and urban lands, the majority of misfits and transients live in private and shared facilities. When it comes to Coronavirus Disease 2019, they suffer the same health risks as their host populations (COVID-19). Outcasts and vagrants may be particularly susceptible due to their brief excursions, limited business hours, crowded and unsuitable living and working circumstances, and lack of access to food, water, sanitation, and other basic necessities. Many homeless people are routinely excluded from national health improvement, sickness prevention, treatment, and care activities, as well as health and social service financial security strategies. This avoidance makes early detection, testing, diagnosis, contact tracing, and obtaining care for COVID-19 difficult for evacuees and their families.

Conclusion
COVID-19's unprecedented problems have impacted society in a variety of ways, including psychological, economical, and environmental issues, as well as health and migration-related issues, resulting in calamitous changes in the worldwide ecosystem. This research focuses on the action mechanism of COVID-19 on the human body, the immunological response to COVID-19, clinical manifestations, and COVID-19 susceptibility prevention methods. COVID-19 has also been studied in terms of diagnostic methods for identification and therapy options. In addition, concerns concerning migrants during a pandemic are discussed in order to enhance the results of COVID-19 cases.

Conflicts of Interest
Authors declared they have no conflicts of interest.

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